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14. ABSTRACT We discovered that germline BAP1 mutations cause a novel cancer syndrome characterized by a very high incidence of malignant mesothelioma (MM). We have conducted a number of in vitro and in vivo experiments to study the mechanism(s) during the two years and obtained exciting results. We found that BAP1 status regulate NF-kB activity and HMGB1 release. BAP1 silenced HM cells (and macrophages) release more HMGB1 into the extra cellular space, which suggests that germline BAP1 mutations by increasing the release of HMGB1 create an environment favorable to malignant transformation. Moreover, we found that BAP1 silenced HM cells are much less sensitive to asbestos induced cytotoxicity, and that BAP1 silenced HM cells form significantly more foci in tissue culture compared to cells containing wild type BAP1. Together these in vitro studies suggested that germline BAP1 mutations would increase susceptibility to asbestos carcinogenesis, which was further proven correct in the animal experiments. We found that BAP1 ^{+/-} mice develop more MMs and had shorter survival (probably related to earlier tumor development) compared to wild type littermates. BAP1 loss increased the susceptibility to low doses of asbestos that rarely cause MM in animals carrying wild type BAP1. Moreover, we linked the increased susceptibility of BAP1 ^{+/-} mice to asbestos carcinogenicity to differences in the chronic inflammatory response, and to the release of specific cytokines and chemokines that follows asbestos exposure in BAP1 ^{+/-} mice.					
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Introduction

Malignant mesothelioma (MM) is an aggressive tumor, which arises from the cells of the mesothelium, the protective lining that covers the lungs and many other internal organs. MM is associated with asbestos exposure. In spite of the stringent regulations that were introduced in the 1970s and 80s to limit asbestos exposure, the incidence of MM has reached 3,200 cases per year in the US in 2003 and it has remained stable since then, while it continues to increase worldwide. We have previously reported that germline BAP1 mutations cause a novel cancer syndrome characterized by high incidence of MM, uveal melanoma, cutaneous melanoma and other cancers. Our findings suggested to us the hypothesis that these individuals may be unusually susceptible to a modest amount of asbestos exposure that is not associated with an overall increase of MM within the general population. In order to study the mechanism(s) by which mutated BAP1 causes MM and whether mutated BAP1 influences factors critical for MM pathogenesis, we conducted a number of in vitro and in vivo experiments and obtained quite exciting results during the two years.

Keywords:

mesothelioma, BAP1, asbestos, mechanisms

Accomplishments:

What were the major goals of the project?

The major goals of the project are to study the mechanisms of how BAP1 mutations predispose individuals to mesothelioma and to find out whether BAP1 mutations increase susceptibility to asbestos carcinogenesis.

What was accomplished under these goals?

To assess the impact of BAP1 on the regulation of pathways critical for the response of HM cells to asbestos fibers, as proposed in Aim 1, we performed experiments using human primary mesothelial cells (HM) that are “knocked down” for BAP1 expression using specific BAP1 siRNA. We found that BAP1 status influences NF-κB activity at basal level as well as upon TNF-α treatment (Figure 1).

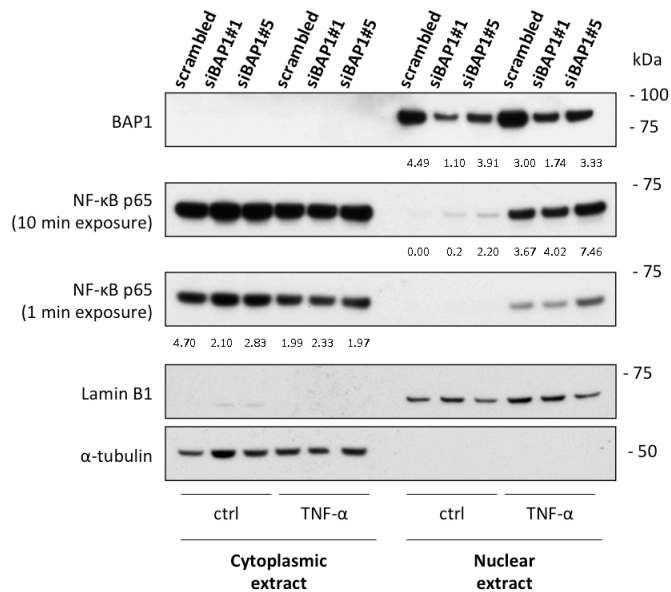


Figure 1. BAP1 silencing in HM increases NF-κB p65 nuclear levels and its translocation after TNF-α treatment. Primary human mesothelial cells (HM) were seeded in 20% FBS media and then mock- (scrambled) or siRNAs-BAP1 (siBAP1#1 and siBAP1#5) transfected for 24 hours in 1% FBS media. Subsequently, HM cells were treated with 10 ng/ml TNF-α, or vehicle as control, in 1% FBS media for 1 hour. Cell fractionation was performed to isolate nuclear and cytoplasmic fraction. Lamin B1 (nuclear extract) and α-tubulin (cytoplasmic extract) were used as loading controls. Numbers indicate relative densitometry units normalized on Lamin B1 for nuclear extract, and α-tubulin for cytoplasmic extract.

To assess the impact of BAP1 in the process of asbestos-induced human mesothelial cells (HM) transformation *in vitro*, we performed in vitro HM transformation assay using our established tissue culture system. We found that by knocking down BAP1 expression using siRNA, HM underwent morphological transformation and developed anchorage independent growth, as well as formed a higher number of tridimensional foci compared to control HM containing wild type BAP1 (Figure 2).

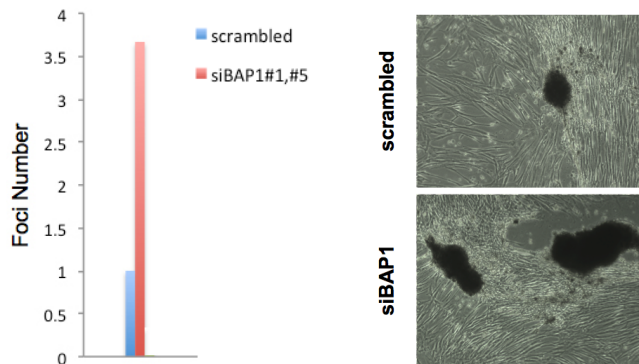


Figure 2. Increased foci formation was observed in HM following BAP1 silencing. HM transfected with mock-(scrambled) or siRNA-BAP1 (siRNA#1 and siBAP1#5) were exposed to asbestos at 5 $\mu\text{g}/\text{cm}^2$ and were cultured for up to 2 months for observation of foci formation. Left panel, foci number; right panel, foci images.

We studied cell growth and cell death in asbestos-exposed HM with or without BAP1 expression. Moreover, we measured HMGB1 release in HM in which we knocked down BAP1 compared to control HM containing wild type BAP1. We found that knocking down BAP1 using siRNA led to increased HMGB1 release into the cell culture media (Figure 3). We further tested macrophages and found that macrophages in which we knocked down BAP1 using siRNA also released more HMGB1 compared to macrophages containing wild type BAP1 (Figure 4).

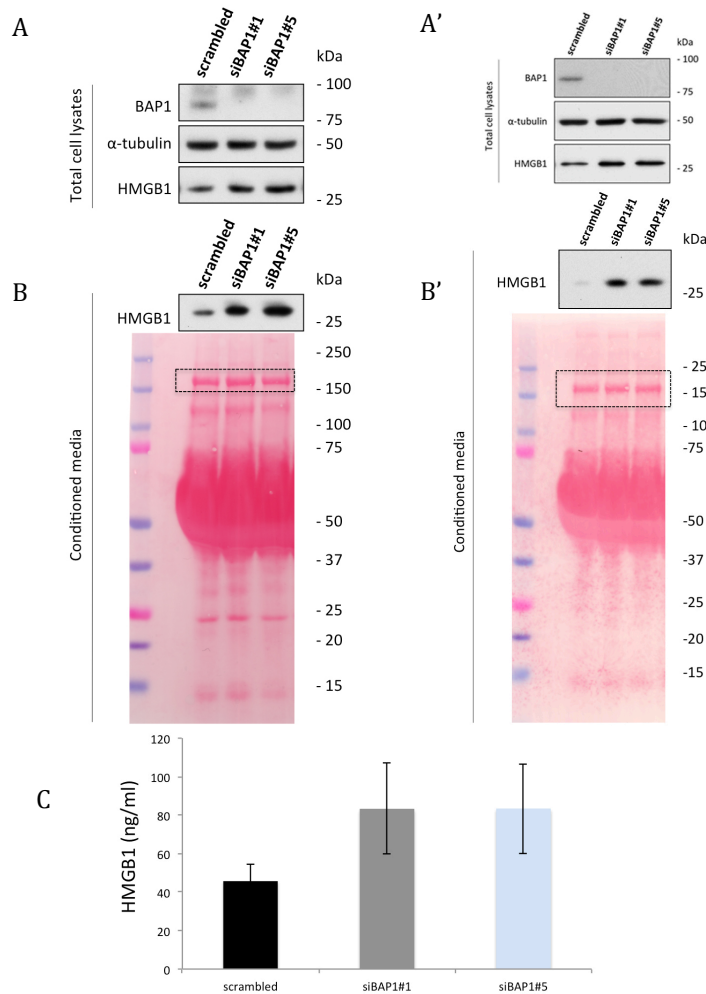


Figure 3. HM cells release more HMGB1 into extra cellular space after BAP1 silencing. (A and A' represent two separate experiments). Total protein was extracted from HM transfected with mock- (scrambled) or siRNA-BAP1 (siRNA#1 and siBAP1#5) and were analyzed by Western blot. (B and B') Conditioned cell culture media were concentrated with Amicon centrifugal filter (Millipore) and then were analyzed by Western blot. (lower panel is the Ponceau staining of the gel showing equal loading). (C) HMGB1 concentrations in the concentrated cell culture media were analyzed by ELISA assay. Experiments were done in triplicate.

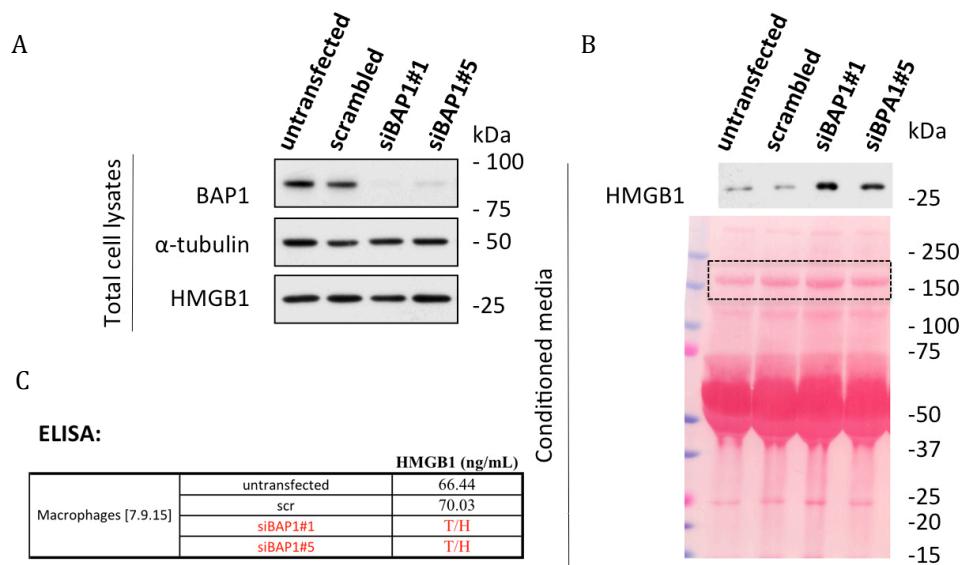


Figure 4. Macrophages release more HMGB1 into the extra cellular space after BAP1 silencing.

(A) Total protein was extracted from macrophages transfected with mock- (scrambled) or siRNA-BAP1 (siRNA#1 and siBAP1#5) and were analyzed by Western blot. (B) Cell culture media were concentrated and analyzed by Western blot. (C) HMGB1 concentrations in the concentrated cell culture media were analyzed by ELISA assay. (Note: The concentrations of HMGB1 in the concentrated media of macrophages transfected with siBAP1#1 and siBAP1#5 were very high and exceeded the detection range of the ELISA kit.

We observed that when we silenced BAP1 in HM, these cells were more resistant to asbestos induced cytotoxicity (Figure 5 A and B), and, accordingly we found that more HM survived after asbestos exposure when BAP1 was silenced. Moreover, although BAP1 silencing induced more HMGB1 release in normal culture condition- as we show above (Figs 3 and 4), HM with silenced BAP1 exposed to asbestos released less HMGB1 compared to control HM containing wild type BAP1 (Figure 5C).

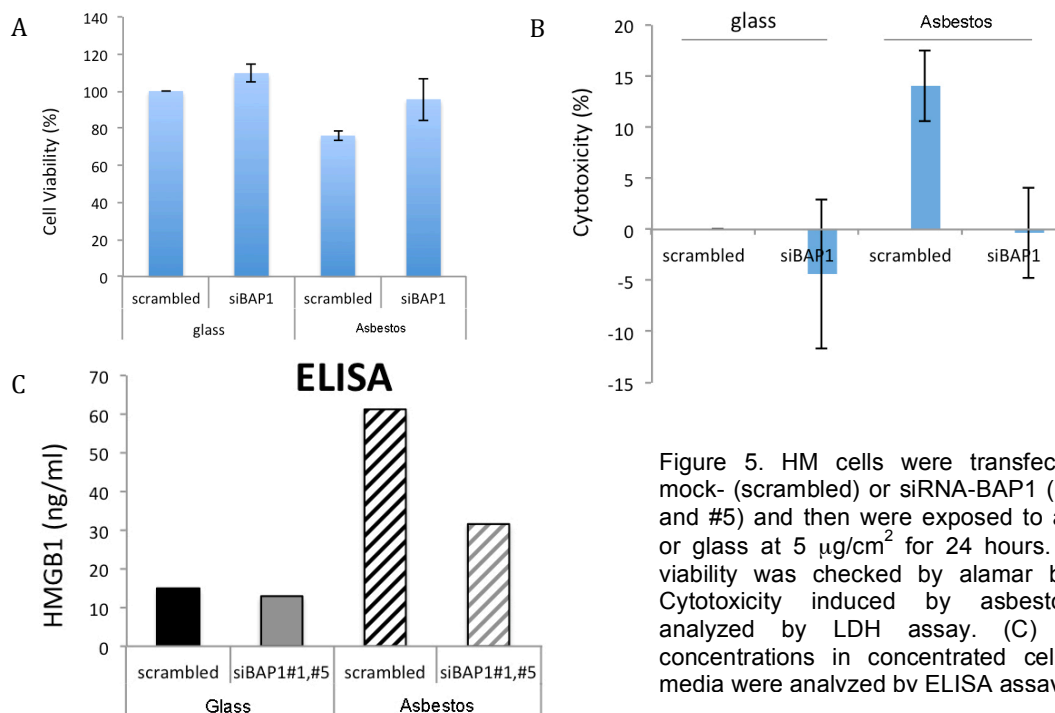


Figure 5. HM cells were transfected with mock- (scrambled) or siRNA-BAP1 (siRNA#1 and #5) and then were exposed to asbestos or glass at $5 \mu\text{g}/\text{cm}^2$ for 24 hours. (A) Cell viability was checked by alamar blue. (B) Cytotoxicity induced by asbestos was analyzed by LDH assay. (C) HMGB1 concentrations in concentrated cell culture media were analyzed by ELISA assay.

To assess the impact of BAP1 in the process of MM development and progression *in vivo*, we performed animal experiment using transgenic mice with heterozygous BAP1 knockout (BAP1^{+/-} mice). We found that the incidence of MM was significantly higher in BAP1^{+/-} mice exposed to asbestos compared to BAP1^{+/+} wild type mice and that mesotheliomas in BAP1^{+/-} mice developed with a reduced latency. Moreover, we observed dramatic difference in MM incidence in mice receiving low doses of asbestos (36% in BAP1 ^{+/-} mice vs. 8% in BAP1 ^{+/+} wild type mice) (Figure 6).

The results are very significant and are exactly as we had anticipated in our grant proposal. We performed histological evaluation of tumors. The results are reported in below and also in our recently published manuscript (Napolitano A, et al. *Oncogene* 2015).

We also compared the profiles of cytokines and chemokines present in peritoneal lavages of BAP1 ^{+/-} and wild type mice injected with asbestos. Compared to wild type littermates, the levels of monocyte chemoattractant protein-1 (MCP-1) were significantly lower in BAP1^{+/-} mice exposed to glass (2.5 pg/mL [2.3-5.2] vs 33.6 pg/mL [6.5-51.7], $P < 0.01$) and in BAP1^{+/-} mice exposed to asbestos (52.4 pg/mL [4.7-113.4] vs 178.5 pg/mL [102.9-373.2], $P < 0.05$). Analogously, compared to wild type littermates, the levels of leukemia inhibitory factor (LIF) were significantly lower in the BAP1^{+/-} mice exposed to glass (0.9 pg/mL [0.9-1.0] vs 6.9 pg/mL [1.1-13.5], $P < 0.01$), and in the BAP1^{+/-} mice exposed to asbestos (78.2 pg/mL [41.0-134.4] vs 201.9 pg/mL [116.9-274.8], $P < 0.05$). Moreover, lavages from BAP1^{+/-} mice exposed to asbestos contained significantly lower amounts of keratinocyte-derived chemokine (KC) compared to wild type littermates (253.4 pg/mL [19.5-557.1] vs 675.3 pg/mL [469.8-1741.5], $P < 0.05$). We also observed that eotaxin levels were significantly lower in BAP1^{+/-} mice compared to wild type littermates in the glass exposed control group (1.73 ng/mL [1.11-2.06] vs 3.27 ng/mL [1.94-3.92], $P < 0.05$); the same trend, although non-significant, was retained following asbestos exposure (3.33 ng/mL [2.56-4.33] vs 4.70 ng/mL [3.13-6.30], $P = 0.28$). Levels of IL-6 also differed between genotypes upon asbestos exposure, though this difference did not reach nominal significance ($P = 0.08$). Both IL-6 and LIF belong to the IL-6 family of cytokines, and in our samples their levels significantly correlated ($R^2 = 0.62$, $P < 0.0001$) (Figure 7). Finally, levels of G-CSF, IL-5, IP-10, and VEGF significantly increased after asbestos exposure, independently of the genotype (Figure 8). Levels of several other cytokines were below the lower limit of detection of our assay. Together, these results indicated that germline BAP1 heterozygosity significantly influenced the peritoneal inflammatory response upon asbestos exposure.

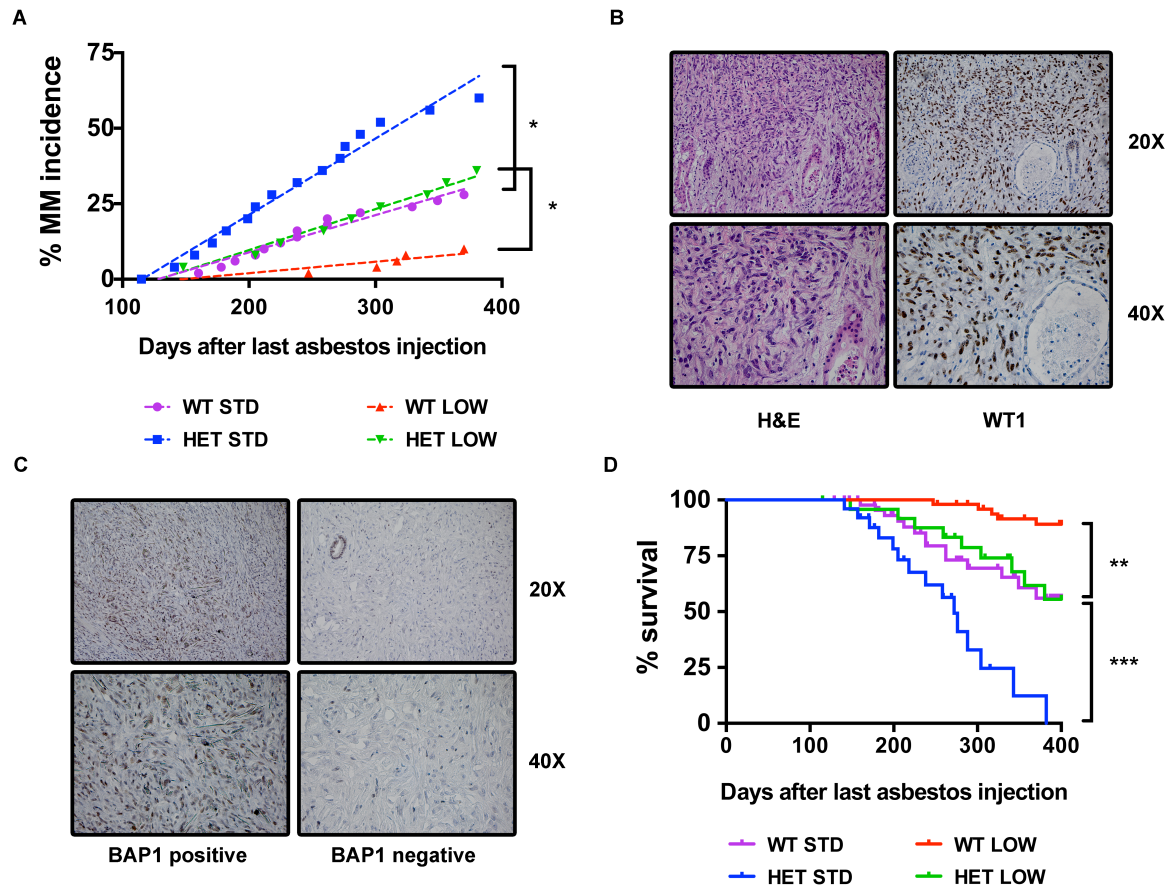


Figure 6. BAP1^{+/-} mice develop more MMs and have shorter survival compared to wild type littermates. Briefly, BAP1^{+/+} mice (WT) (n = 50 per group) and BAP1^{+/-} mice (HET) (n = 25 per group) were injected intraperitoneally every week for ten weeks with 0.05 mg (low dose, LOW) or 0.5 mg (standard dose, STD) of asbestos. 0.5 mg of glass beads were injected at the same schedule as control. (A) MM incidence in BAP1^{+/-} mice and wild type littermates after long-term exposure to glass beads or asbestos fibers (standard and low dose) was compared using Fisher's exact test. * ($P < 0.05$) (B) Formalin-fixed/paraffin-embedded samples were stained with Hematoxylin and Eosin (H&E) according to standard procedure. The pathological diagnosis of mesothelioma was based on H&E staining and supported by WT1 nuclear staining in tumor cells. H&E and immunostainings were blindly interpreted by two US board specialized pathologists with expertise in human and animal mesotheliomas (C) Tumors were also stained with a rabbit polyclonal anti-BAP1 antibody to evaluate presence and localization of BAP1. (D) Survival curves of BAP1^{+/-} mice and wild type littermates after long-term exposure to asbestos fibers (standard and low dose) were compared using log-rank (Mantel-Cox) test. ** ($P < 0.01$), *** ($P < 0.001$).

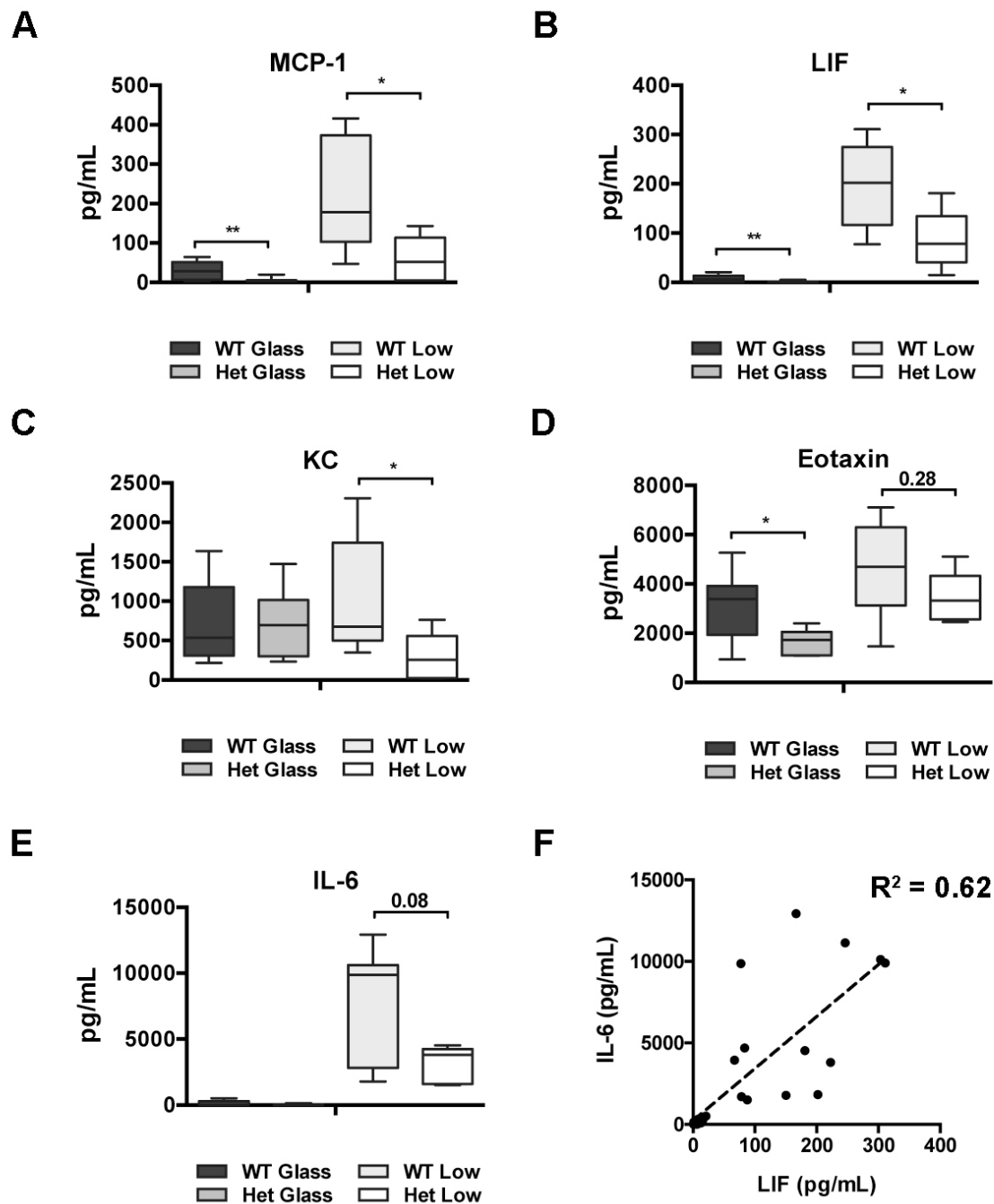


Figure 7. Several cytokines and chemokines are differentially expressed in lavage from BAP1^{+/-} mice following asbestos exposure. The supernatants recovered from the peritoneal lavages were concentrated 45-60 times using Amicon Ultra Centrifuge Filters with a 3,000 Dalton cutoff. Levels of 32 cytokines and chemokines were detected in concentrated lavages using human cytokine multiplex kits (EMD Millipore Corporation, Billerica, MA). Levels of MCP-1 (A), LIF (B), KC (C), eotaxin (D) and IL-6 (E) in lavages from BAP1 wild type and heterozygous mice after short-term exposure to glass beads or asbestos fibers. Comparisons between heterozygous and wild type groups were calculated using Mann-Whitney U test for rank comparisons. * ($P < 0.05$), ** ($P < 0.01$) (F) Correlation of IL-6 and LIF levels (both belonging to the IL-6 family of cytokines) calculated using linear regression. The experiment was replicated two times.

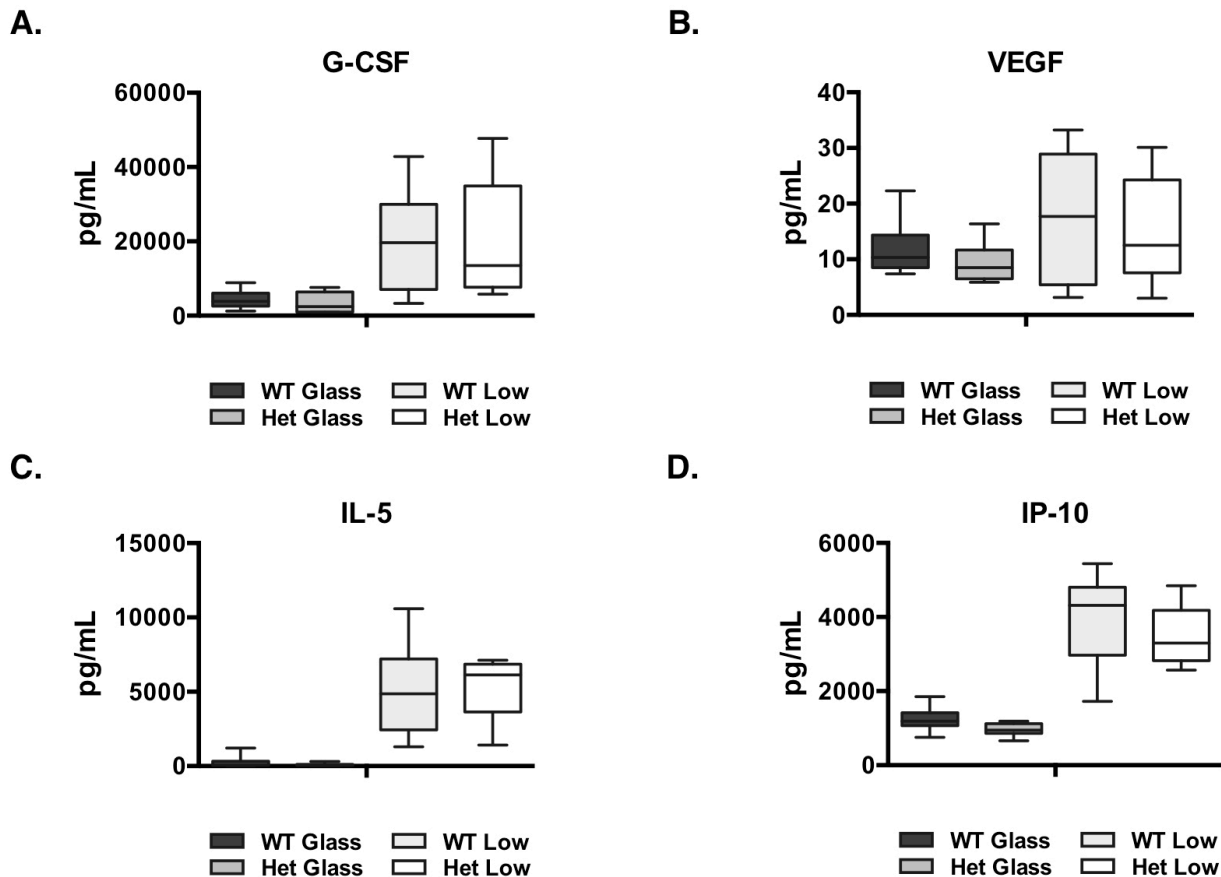


Figure 8. Levels of other cytokines and chemokines are not differentially expressed.

(A), G-CSF (B), VEGF (C), IL-5 (D) IP-10 in lavages from BAP1 wild type and heterozygous mice after short-term exposure to glass beads or asbestos fibers. Comparisons between heterozygous and wild type groups were calculated using Mann-Whitney U test for rank comparisons. No statistically significant differences were observed. The experiment was replicated two times

In summary, we discovered that BAP1^{+/-} mice exposed to low doses of asbestos developed MMs at a similar rate as BAP1^{+/+} mice exposed to 10 times higher doses. Therefore, although it is not possible to directly compare the low-dose exposure in mice to indoor and/or outdoor environmental exposure in humans, our findings support our hypothesis that germline BAP1 heterozygosity increases susceptibility to the carcinogenic effects of low doses of asbestos. Moreover, our results suggest a novel, complex model of asbestos-induced MM pathogenesis, in which the chronic inflammatory response can have preferentially anti-tumoral or pro-tumoral roles, depending on the cellular and soluble mediators involved.

Next, we detected the levels of HMGB1 after asbestos exposure in these BAP1^{+/-} and BAP1^{+/+} mice to further understand the role of HMGB1 in the development of MM. We found that three days after intraperitoneal injection of crocidolite asbestos, although both BAP1^{+/-} and BAP1^{+/+} mice had significantly elevated levels of HMGB1 in peritoneal lavage fluid after asbestos injection compared to control glass injection, lower levels of HMGB1 were found in BAP1^{+/-} mice compared to BAP1^{+/+} mice (Figure 9). In order to understand the reason why BAP1^{+/-} mice had lower levels of HMGB1 in peritoneal lavage, we extracted mouse mesothelial cells from BAP1^{+/-} and BAP1^{+/+} mice and treated them with crocidolite asbestos. We found that mesothelial cells from BAP1^{+/-} mice are more resistant to asbestos-induced cell death, in comparison to the mesothelial cells from BAP1^{+/+} mice.

This is similar to what we observed in engineered human cells --HM containing different BAP1 expression status as shown in Figure 5. We then conducted cytotoxicity LDH assay (Figure 10A) and cell viability Alamar blue assay (Figure 10B) to confirm these findings. Our results support that there were less BAP1^{+/-} mouse mesothelial cells died after asbestos exposure compared to BAP1^{+/+} mesothelial cells. Moreover, we found that bone marrow-derived macrophages (BMDMs) from BAP1^{+/-} mice release similar levels of HMGB1 as BMDMs from BAP1^{+/+} mice (Figure 11). These findings further suggest that the lower levels of HMGB1 detected in the peritoneal lavage of BAP1^{+/-} mice were due to less HMGB1 passively release from dying mesothelial cells.

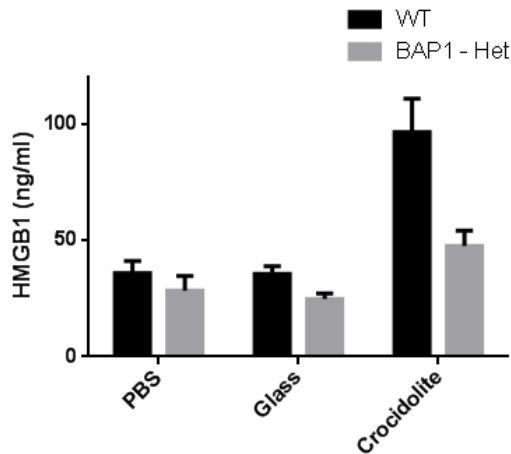


Figure 9. Less HMGB1 is found in peritoneal lavage fluid of BAP1^{+/-} mice (BAP1-Het) compared to BAP1^{+/+} mice (WT) after intraperitoneal injection of crocidolite asbestos. HMGB1 levels were measured by ELISA assay in peritoneal lavage fluid in BAP1^{+/+} and BAP1^{+/-} mice three days after injection of crocidolite asbestos, glass control or PBS control.

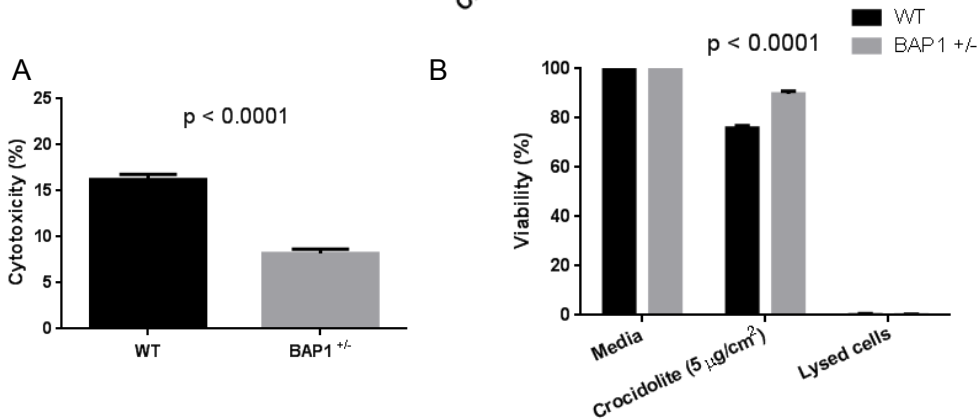


Figure 10. BAP1^{+/-} mouse mesothelial cells are resistant to asbestos-induced cell death. Mouse mesothelial cells extracted from BAP1^{+/-} mice and from BAP1^{+/+} mice were cultured and treated with crocidolite at 5 mg/cm². Cell cytotoxicity was analyzed by LDH assay (A), and cell viability was detected by Alamar Bjué assay (B).

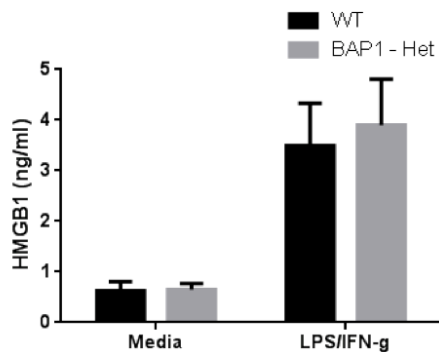
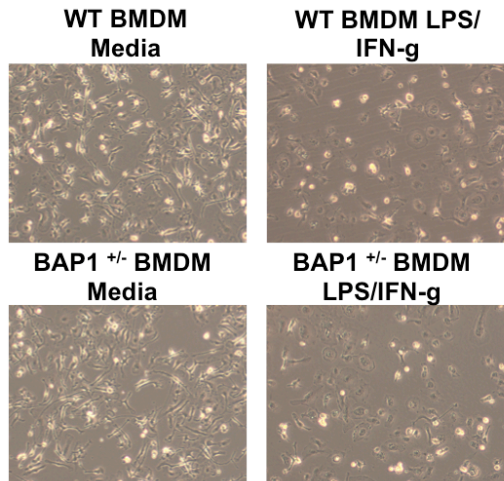


Figure 11. Bone marrow-derived macrophages (BMDMs) from BAP1^{+/-} mice release similar levels of HMGB1 as WT BAP1^{+/+} mice. Bone marrow was harvested from WT and BAP1^{+/-} mice and was cultured for 5 days with 20 ng/mL m-CSF in 10% RPMI. Bone marrow macrophages were then stimulated for 2 days with media or media supplemented with IFN-γ (100 ng/ml) and LPS (50 ng/ml). Supernatants were collected and HMGB1 levels measured by ELISA.

Key Research Accomplishments:

- (I) BAP1 status influences NF- κ B activity at basal level as well as upon TNF- α treatment.
- (II) BAP1 silencing induces more foci formation in HM cells exposed to asbestos.
- (III) Both HM and macrophages cells release more HMGB1 into the extra cellular space following BAP1 silencing.
- (IV) HM cells with silenced BAP1 are more resistant to asbestos induced cytotoxicity, therefore less HM die: consequently, the amount HMGB1 passively released by dying cells following asbestos exposure, and measured in the tissue culture medium, is decreased in cells carrying BAP1 mutations compared to cells with wild type BAP1.
- (V) BAP1 $^{+/-}$ mice develop more MMs and have shorter survival compared to wild type littermates. Moreover, BAP1 germline mutations increase the susceptibility to low dose of asbestos and mesothelioma.
- (VI) Several cytokines and chemokines are differentially expressed in lavage from BAP1 $^{+/-}$ mice following asbestos exposure, in particular MCP-1, LIF, KC, eotaxin and IL-6, suggesting that they play an important role in the mechanisms responsible for the increased susceptibility of BAP1 $^{+/-}$ mice to asbestos and mesothelioma.
- (VII) We further confirmed that BAP1 status influences the levels of HMGB1 in mouse model.

Conclusion:

Our findings suggest that minimal exposure to carcinogenic fibers may significantly increase the risk of malignant mesothelioma in genetically predisposed individuals carrying germline BAP1 mutations. We found that BAP1 can regulate NF- κ B activity as well as HMGB1 release. We have achieved the major goals that were proposed, and our results elucidated some of the mechanisms of how BAP1 loss influences the cellular responses to asbestos and provide a rationale for the increased the susceptibility of carriers of BAP1 $^{+/-}$ mutations to asbestos and MM. Moreover, we linked the increased susceptibility of BAP1 $^{+/-}$ mice to asbestos carcinogenicity to differences in the chronic inflammatory response, and to the release of specific cytokines and chemokines that follows asbestos exposure in BAP1 $^{+/-}$ mice.

What opportunities for training and professional development has the project provided?

I attended various meetings including AACR, iMig meeting and attended meetings organized by Mesothelioma Applied Research Foundation during the two years, and we gave presentation and seminars quite a few times. I have gained professional skills and experience in mesothelioma research. I was promoted to Tenured Associate Professor. And also, the data and experience I gained during these years helped me win another two grants recently from DoD, one is the Idea Award and the other one is the Team Award.

How were the results disseminated to communities of interest?

Our research findings disseminate to communities of interest. Our findings suggest that minimal exposure to carcinogenic fibers may significantly increase the risk of malignant mesothelioma in genetically predisposed individuals carrying germline BAP1 mutations. Thus we proposed that carriers of germline BAP1 mutations should avoid jobs in trades were even minimal exposure to asbestos may occur –such as mechanics, electricians, and certain military branches, such as military working on ships and submarines.

Our suggestion that BAP1 mutant carriers should avoid trades in which a minimal exposure to asbestos – i.e., an amount of exposure that would not be considered to increase mesothelioma risk among the population at large- may occur, was reviewed and agreed upon by a board of experts and has now been reported as suggestive guideline for carriers of BAP1 mutations.

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to report. This is the final report.

IMPACT:**What was the impact on the development of the principal discipline(s) of the project?**

The exact mechanisms of how asbestos causes MM are being elucidated but remain unclear. Our findings on asbestos carcinogenesis and the contributory role of genetics (BAP1) provide us a novel opportunity to elucidate the key mechanisms of asbestos carcinogenesis and MM, and to subsequently develop targeted therapies for this very aggressive disease.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

The results from this project are likely to make an impact on public knowledge regarding mesothelioma pathogenesis. It will make more people aware of that minimal exposure to carcinogenic fibers may significantly increase the risk of malignant mesothelioma in genetically predisposed individuals carrying germline BAP1 mutations, and that individuals carrying germline BAP1 mutations should avoid even minimal exposure to asbestos or other carcinogenic fibers.

CHANGES/PROBLEMS:

Nothing to report.

Products:**Publications, Abstracts, and Presentations:***(I) Peer-Reviewed Scientific Journals:*

1. Comertpay S, Pastorino S, Tanji M, Mezzapelle R, Strianese O, Napolitano A, Baumann F, Weigel T, Friedberg J, Sugarbaker P, Krausz T, Wang E, Powers A, Gaudino G, Kanodia S, Pass HI, Parsons BL, Yang H, Carbone M. Evaluation of clonal origin of malignant mesothelioma. *J Transl Med*. 2014. Dec 4;12:301. PMID: 25471750
2. Baumann F, Flores E, Napolitano A, Kanodia S, Taioli E, Pass H, Yang H, Carbone M. Mesothelioma patients with germline BAP1 mutations have 7-fold improved long-term survival. *Carcinogenesis*. 2015 Jan;36(1):76-81. PMID: 25380601
3. Nasu M, Emi M, Pastorino S, Tanji M, Powers A, Baumann F, Zhang Y, Gazdar A, Kanodia S, Tiirikainen M, Flores E, Gaudino G, Becich MJ, Pass HI, Yang H and Carbone M. High incidence of somatic BAP1 alterations in sporadic malignant mesothelioma. *J Thorac Oncol*. 2015 Apr;10(4):565-76. PMID: 25658628.
4. Carbone M, Gaudino G, Yang H. Recent insights emerging from malignant mesothelioma genome sequencing. *J Thorac Oncol*. 2015 Mar;10(3):409-11. PMID: 25695218
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6. Napolitano A, Pelegri L, Anwesha D, Larson D, Tanji M, Flores EG, Kendrick B, Lapid D, Powers A, Kanodia S, Pastorino S, Pass HI, Dixit V, Yang H and Carbone M. Minimal asbestos exposure in germline BAP1 heterozygous mice is associated with deregulated inflammatory response and increased risk of mesothelioma. *Oncogene*. 2015 Jun 29. (Epub ahead of print) PMID: 26119930.
7. Bononi A, Napolitano A, Pass HI, Yang H, Carbone M. Latest developments in our understanding of the pathogenesis of mesothelioma and the design of targeted therapies. *Expert Rev Respir Med*. 2015 Oct;9(5):633-54. PMID: 26308799
8. Carbone M, Chao A, Kanodia S, Miller A, Wali A, Weissman D, Adjei A, Baumann F, Boffetta P, Buck B, Perrot M, Dogan AU, Gavett S, Gualtieri A, Hassan R, Hesdorffer M, Hirsch FR, Larson D, Mao W, Masten S, Pass HI, Peto J, Pira E, Steele I, Tsao A, Woodard GA, Yang H and Malik S.

(II) Invited Articles:

1. Napolitano A, Jube S, Gaudino G, Pass HI, Carbone M and **Yang H**. Asbestos-induced chronic inflammation and cancer. In: Cancer and Inflammation Mechanisms: Chemical, Biological, and Clinical Aspects. Shousuke Kawanishi, Hiroshi Ohshima and Yusuke Hiraku (Ed.) 2014. ISBN: 978-1-118-16030-5

(III) Meeting Presentations:

1. Nasu M, Napolitano A, Pastorino S, Tanji M, Flores E, Baumann F, Powers A, Gaudino G, Pass HI, **Yang H**, Carbone M. BAP1 mutation in mesothelioma and "BAP1 Cancer Syndrome". 2014 AACR Annual Meeting. April 5-9, 2014. San Diego, CA, USA.
2. Nasu M, Napolitano A, Pastorino S, Tanji M, Flores E, Baumann F, Powers A, Gaudino G, Pass HI, **Yang H**, Carbone M. BAP1 mutation in mesothelioma and "BAP1 Cancer Syndrome". Weinman Symposium, UH Cancer Center, May 2nd, 2014. Honolulu, HI, USA.
3. Napolitano A, Pelegri L, Anwesh D, Larson D, Tanji M, Flores EG, Kendrick B, Lapid D, Powers A, Kanodia S, Pastorino S, Pass HI, Dixit V, Yang H and Carbone M. Germline BAP1 heterozygous mice are sensitive to low dose of asbestos and have increased risk of mesothelioma. AACR 2015

I was invited to give talks at several National and International meetings, where I presented (or will present) these data:

1. University of Ferrara. 2015, April, Ferrara, Italy.
2. New Frontiers in Oncology. 2015, April, Rome, Italy.
3. Mt Sinai Hospital. 2015, June. New York, NY.
4. Magna Graecia University and Tommaso Campanella Cancer Center. 2015, September. Catanzaro, Italy.
5. 7th International Symposium DAMPS and HMGB. 2015, September. Bonn, Germany.
6. Weinman International Conference on Mesothelioma, Honolulu, Hawaii, Nov 2015.
7. University of Torino. 2015, November, Turin, Italy.
8. International Mesothelioma Interest Group. Birmingham, UK May 1-4, 2016

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Haining Yang (PI)

David Larson (Postdoc Fellow) "No change"

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

SPECIAL REPORTING REQUIREMENTS

Nothing to report.

APPENDICES:

SHORT COMMUNICATION

Minimal asbestos exposure in germline *BAP1* heterozygous mice is associated with deregulated inflammatory response and increased risk of mesothelioma

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Germline *BAP1* mutations predispose to several cancers, in particular malignant mesothelioma. Mesothelioma is an aggressive malignancy generally associated with professional exposure to asbestos. However, to date, we found that none of the mesothelioma patients carrying germline *BAP1* mutations were professionally exposed to asbestos. We hypothesized that germline *BAP1* mutations might influence the asbestos-induced inflammatory response that is linked to asbestos carcinogenesis, thereby increasing the risk of developing mesothelioma after minimal exposure. Using a *BAP1*^{+/-} mouse model, we found that, compared with their wild-type littermates, *BAP1*^{+/-} mice exposed to low-dose asbestos fibers showed significant alterations of the peritoneal inflammatory response, including significantly higher levels of pro-tumorigenic alternatively polarized M2 macrophages, and lower levels of several chemokines and cytokines. Consistent with these data, *BAP1*^{+/-} mice had a significantly higher incidence of mesothelioma after exposure to very low doses of asbestos, doses that rarely induced mesothelioma in wild-type mice. Our findings suggest that minimal exposure to carcinogenic fibers may significantly increase the risk of malignant mesothelioma in genetically predisposed individuals carrying germline *BAP1* mutations, possibly via alterations of the inflammatory response.

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INTRODUCTION

Malignant mesothelioma (MM) is a deadly cancer usually localized to the pleural and peritoneal linings.¹ In the US and in the UK, ~3200 and ~2500 individuals are diagnosed with and die because of MM each year, respectively.^{2,3} About 60–70% of mesotheliomas have been associated with exposure to carcinogenic mineral fibers, mainly asbestos.¹ Nevertheless, the risk of developing MM in high-risk cohorts professionally exposed to asbestos is ~5%, suggesting that other factors contribute to MM pathogenesis.¹ Mineral fibers promote mesothelioma inducing a chronic inflammatory reaction: on one hand, this results in the production of mutagenic oxygen and nitrogen radicals, and on the other hand, it provides damaged mesothelial cells with important survival signals.⁴ Although chronic inflammation has been associated with the pathogenesis of several cancers, competent inflammatory cells also provide immunosurveillance, the host's protection process against nascent transformed cells expressing altered antigens.⁵ In fact, different functional and phenotypical cell subtypes are associated to anti-tumoral or pro-tumoral immunity.⁶ Macrophages (MΦ) can undergo different types of polarization based on the kind and levels of cytokines present in the local tissue environment. Classically activated (M1) MΦ have a pro-inflammatory anti-tumoral phenotype, whereas alternatively activated (M2) MΦ are involved in immunosuppression and tissue repair.⁷ Tumor-associated macrophages represent one of the major populations of immune cells infiltrating tumors, and usually acquire functional

characteristics similar to M2 MΦ.⁸ The ratio between M2-like and M1-like tumor-associated macrophages has prognostic value in MM and other cancers, with the former usually associated with a worse prognosis.^{9–11} However, the contribution of different MΦ subpopulations to the initiation of inflammation-induced cancers is still unclear. MM has a large number of tumor-associated macrophages, suggesting that they have an important role in this malignancy.¹²

Recently, we identified germline mutations in the tumor suppressor gene *BRCA1 associated protein-1* (*BAP1*) as causative of a novel hereditary cancer syndrome characterized by a very high risk of MM, uveal and cutaneous melanoma, several other malignancies and characteristic benign melanocytic tumors we named MBAITs.^{13–15} The penetrance of the *BAP1* cancer syndrome is ~100%, and several patients carrying germline *BAP1* mutations develop multiple cancers.¹⁶ Notably, none of the germline *BAP1* heterozygous patients who developed MM reported professional exposure to asbestos fibers,^{13,16} suggesting that either these MMs were not caused by asbestos or that minimal amounts of asbestos—as in the case of some indoor exposure¹⁷ or naturally occurring outdoor environmental exposure¹⁸—may be sufficient to cause MM in germline *BAP1* mutation carriers. Here, we experimentally tested in a *BAP1*^{+/-} murine model whether germline *BAP1* heterozygosity would result in alterations of the asbestos-induced inflammatory response, and whether low doses of asbestos might be sufficient to cause MM.

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We used constitutive BAP1^{+/-} mice (C57BL/6 background) generated by breeding mice with *loxP* sites flanking BAP1 exons 4 and 5 with mice expressing a constitutive general Cre deleter.¹⁹ Although homozygous BAP1 deficiency in mice results in embryonic lethality,¹⁹ BAP1^{+/-} mice are viable and healthy. Compared with wild-type littermates, BAP1^{+/-} mice expressed about half the amount of BAP1 protein in relevant tissues (Supplementary Figure 1).

In our experiments, we used 10–12-week-old mice of either sex equally distributed in the experimental groups using a computational random number generator. All the experiments were approved by the University of Hawaii Institutional Animal Care and Use Committee (IACUC). Unless otherwise specified, results are presented as median (interquartile range).

RESULTS

First, we exposed BAP1^{+/-} mice and BAP1^{+/+} for 5 weeks to injections with glass beads or a low amount of crocidolite asbestos fibers (0.05 mg/week). After performing a peritoneal lavage, we counted the total number of peritoneal cells and determined via flow cytometry the percentage of total and subset-specific leukocytes. CD45+ leukocytes represented 95–99% of the total cells recovered in each group. In the glass control groups, macrophages and B cells represented the most abundant population, regardless of genotype (Table 1). Upon exposure to low-dose crocidolite fibers, the cellular inflammatory response was largely overlapping in mice with either genotype. We observed a significant increase in the total number of leukocytes and in the relative percentage of neutrophils, and, at the same time, a significant decrease in the percentage of B cells and macrophages (Table 1). Further characterization of the cell types revealed that exposure to crocidolite fibers induced significant alterations in macrophage polarization in BAP1^{+/-} mice (Figure 1a). In the macrophages from BAP1^{+/-} mice exposed to asbestos fibers, the normalized mean fluorescence intensity for CD206 (marker of M2 macrophages) was significantly higher compared with controls (197.1% (160.6–256.8) vs 163.1% (125.4–186.7), $P < 0.05$), whereas the normalized mean fluorescence intensity for CD86 (marker of M1 macrophages) was significantly lower compared with controls (74.6% (57.6–90.3) vs 95.8% (77.4–109.1), $P < 0.05$) (Figure 1b). Accordingly, the percentage of M1 macrophages (CD206 – CD86+ cells) was significantly lower in BAP1^{+/-} mice (43.2% (28.9–44.9) vs 67.3% (46.7–78.2) of total macrophages, $P < 0.05$). On the other

hand, the percentage of M2 macrophages (defined as CD206+ CD86 – cells) was significantly higher in BAP1^{+/-} mice compared with wild-type littermates (3.8% (2.1–6.8) vs 1.2% (0.5–3.6) of total macrophages, $P < 0.05$). Double positive (CD206+ CD86+) macrophages, which represent a transition state from M1 to M2, were also more represented in BAP1^{+/-} mice compared with wild-type littermates (40.0% (30.7–47.0) vs 26.0% (13.3–37.6) of total macrophages, $P < 0.05$) (Figure 1c). Moreover, the M2/M1 ratio (overall percentage of CD206+ cells divided by overall percentage of CD86+ cells) was significantly higher in asbestos-exposed BAP1^{+/-} mice compared with controls (0.54 (0.48–0.66) vs 0.36 (0.16–0.56), $P < 0.05$) (Figure 1d).

Next, we compared the profiles of cytokines and chemokines present in peritoneal lavages of these same mice. Compared with wild-type littermates, the levels of monocyte chemoattractant protein-1 (MCP-1) were significantly lower in BAP1^{+/-} mice exposed to glass (2.5 pg/ml (2.3–5.2) vs 33.6 pg/ml (6.5–51.7), $P < 0.01$) and in BAP1^{+/-} mice exposed to asbestos (52.4 pg/ml (4.7–113.4) vs 178.5 pg/ml (102.9–373.2), $P < 0.05$) (Figure 2a). Analogously, compared with wild-type littermates, the levels of leukemia inhibitory factor were significantly lower in the BAP1^{+/-} mice exposed to glass (0.9 pg/ml (0.9–1.0) vs 6.9 pg/ml (1.1–13.5), $P < 0.01$), and in the BAP1^{+/-} mice exposed to asbestos (78.2 pg/ml (41.0–134.4) vs 201.9 pg/ml (116.9–274.8), $P < 0.05$) (Figure 2b). Moreover, lavages from BAP1^{+/-} mice exposed to asbestos contained significantly lower amounts of keratinocyte-derived chemokine compared with wild-type littermates (253.4 pg/ml (19.5–557.1) vs 675.3 pg/ml (469.8–1741.5), $P < 0.05$) (Figure 2c). We also observed that eotaxin levels were significantly lower in BAP1^{+/-} mice compared with wild-type littermates in the glass-exposed control group (1.73 ng/ml (1.11–2.06) vs 3.27 ng/ml (1.94–3.92), $P < 0.05$); the same trend, although non-significant, was retained following asbestos exposure (3.33 ng/ml (2.56–4.33) vs 4.70 ng/ml (3.13–6.30), $P = 0.28$) (Figure 2d). Levels of interleukin (IL)-6 also differed between genotypes upon asbestos exposure, though this difference did not reach nominal significance ($P = 0.08$) (Figure 2e). Both IL-6 and leukemia inhibitory factor belong to the IL-6 family of cytokines, and in our samples, their levels significantly correlated ($R^2 = 0.62$, $P < 0.0001$) (Figure 2f). Finally, levels of granulocyte colony-stimulating factor, IL-5, IP-10 and vascular endothelial growth factor significantly increased after asbestos exposure, independently of the genotype (Supplementary Figures 2a–d). Levels of several other cytokines were below the lower limit of detection of our assay. Together, these results

Table 1. Major subpopulations of peritoneal leukocytes are not influenced by germline BAP1 heterozygosity

Cells	WT Glass	WT Asb	Het Glass	Het Asb	P value			
					WT (G vs A)	Het (G vs A)	Glass (WT vs Het)	Asb (WT vs Het)
Total leukocytes ($\times 10^6$)	2.7 (1.3–3.6)	6.1 (3.5–14.2)	2.7 (1.3–4.9)	8.5 (4.9–12.7)	< 0.01	< 0.05	ns	ns
Neut (%)	1.8 (1.6–2.4)	13.0 (11.3–16.4)	1.1 (0.8–2.2)	10.4 (9.9–16.6)	< 0.0001	< 0.001	ns	ns
B cells (%)	20.4 (17.5–26.3)	12.7 (9.9–14.2)	19.4 (17.8–21.3)	10.3 (8.6–12.6)	< 0.01	< 0.01	ns	ns
T cells (%)	7.0 (5.1–10.4)	5.0 (3.8–6.4)	6.4 (4.1–10.8)	7.7 (4.3–8.4)	ns	ns	ns	ns
MΦ (%)	33.4 (27.0–38.5)	21.3 (18.6–27.5)	24.2 (20.1–45.2)	19.2 (14.6–22.8)	< 0.01	< 0.05	ns	ns

Abbreviation: WT, wild type. BAP1^{+/-} mice ($n = 7$ per group) and BAP1^{+/+} ($n = 9$ per group) were injected intraperitoneally every week for 5 weeks with 0.05 mg of inert glass beads or crocidolite asbestos fibers, for a total dose of 0.25 mg per mouse. Sample size was estimated hypothesizing a 60% difference in the levels of at least one cytokine. Full mineralogical characterization of crocidolite fibers used in these experiments was reported previously.⁴⁶ Next, mice were killed by CO₂ asphyxiation, and the abdominal cavity was washed with 5 ml of phosphate-buffered saline. The peritoneal cells obtained were pelleted and supernatant was removed for later cytokine analysis. Cells were blindly characterized with the following antibodies: CD45 (leukocytes; anti-CD45-BV711, 563709, BD Biosciences, San Jose, CA, USA), F4/80 (MΦ; anti-F4/80-AlexaFluor488, MCA497A488T, AbD Serotec, Raleigh, NC, USA), Ly-6G (neutrophils; anti-Ly6G-BV421, 562737, BD Biosciences), CD3 (T cells; anti-CD3-APC, 17-0032-80, eBioscience, San Diego, CA, USA) and B220 (B cells; anti-B220-PE, 561878, BD Biosciences). Comparisons between groups were calculated using Mann–Whitney U test for rank comparisons. Results are presented as median (interquartile range).

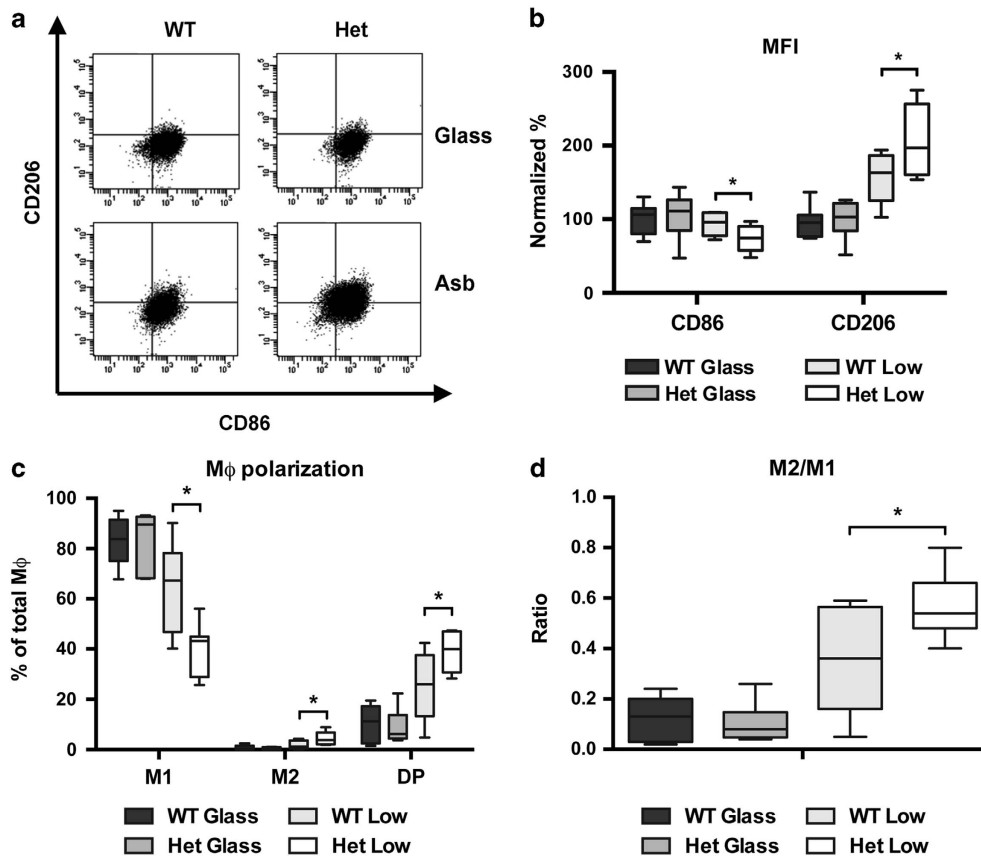


Figure 1. MΦ polarization is altered in $BAP1^{+/-}$ mice exposed to low doses of asbestos fibers. Macrophages and macrophage subtypes were identified using a separate tube of peritoneal cells stained for general MΦ markers CD11b (anti-CD11b-Bv711, 563168, BD Biosciences) and F4/80, CD206 (M2 marker; anti-CD206-APC, 141707, BioLegend, San Diego, CA, USA) and CD86 (M1 marker; anti-CD86-PE, 561963, BD Biosciences). (a) Representative flow cytometry dot plot of peritoneal MΦ in $BAP1^{+/-}$ mice and wild-type littermates after short-term treatment with glass beads or crocidolite asbestos. (b) Mean fluorescence intensities of CD86 and CD206. (c) Percentage of MΦ subpopulations: M1 (CD86+ CD206-), M2 (CD86- CD206+), double positive (DP) (CD86+ CD206+). (d) M2/M1 ratio (overall percentage of CD206+ cells divided by overall percentage of CD86+ cells). Comparisons between heterozygous and wild-type groups were calculated using Mann-Whitney U test for rank comparisons. * $P < 0.05$. The experiment was replicated two times.

indicated that germline *BAP1* heterozygosity significantly influenced the peritoneal inflammatory response upon asbestos exposure.

Therefore, we sought to experimentally study the relationship between asbestos dosage and MM carcinogenesis in the context of *BAP1* heterozygosity. On the basis of previous publications on murine models^{20,21} and on our own experience (Carbone, unpublished observations), doses of asbestos ranging from 3 to 5 mg induce MM in ~20–40% of exposed animals, while 0.5 mg of asbestos induce MM in 0–10% of exposed animals. $BAP1^{+/+}$ mice and $BAP1^{+/-}$ mice received 10 weekly injections of 0.5 mg of crocidolite asbestos fibers (total of 5 mg, referred to as 'standard-dose' as it is the dose most commonly used to induce MM in rodents), 0.05 mg of crocidolite fibers (total of 0.5 mg, referred to as 'low-dose') or 0.5 mg of inert glass beads (total of 5 mg, negative control). During the 13 months of follow-up after the last injection, we did not observe MM or any other spontaneous tumor in the glass control groups. In mice exposed to asbestos fibers, MM was the only malignancy observed. In the low-dose group, crocidolite fibers caused pathologically confirmed MM in 9/25 (36.0%) $BAP1^{+/-}$ mice compared with 5/50 (10.0%) $BAP1^{+/+}$ mice ($P=0.010$). Similarly, in the standard-dose group, MM was diagnosed in 15/25 (60.0%) $BAP1^{+/-}$ mice compared with 14/50 (28.0%) $BAP1^{+/+}$ mice ($P=0.011$) (Figure 3a). Immunohistochemical staining of the tumors revealed expression of the mesothelial

marker WT1 (Figure 3b), supporting the histologic diagnosis of MM. In sporadic human MM, somatic *BAP1* inactivation is one of the most frequent events, and it has been reported in about 40–60% of the cases.^{13,22–27} Consistent with these human data, *BAP1* nuclear staining was absent in all MM analyzed arising from $BAP1^{+/-}$ mice and in 66.7% from $BAP1^{+/+}$ mice (Figure 3c). With regard to histology, all the MMs we observed in human germline *BAP1* mutation carriers were epithelioid.¹³ In sporadic human MMs, several groups have reported that mutations of *BAP1* occur primarily in epithelioid MM,^{24,25} although this is not unequivocal.²⁸ All the MMs we observed in our $BAP1^{+/-}$ and $BAP1^{+/+}$ mice displayed, totally or partially, sarcomatoid features. This is likely due to interspecies differences, because sarcomatoid features, contrary to what happens in human MMs, were also prevalent in MMs arising from other independent murine models of asbestos-induced MM.^{29,30} $BAP1^{+/-}$ mice had also a significantly shorter survival, that is, life span, compared with $BAP1^{+/+}$ mice, both in the low-dose ($P < 0.01$) and the standard-dose group ($P < 0.001$) (Figure 3d).

DISCUSSION

Taken together, our results showed that germline *BAP1* heterozygosity is associated with a significantly altered peritoneal inflammatory response upon exposure to asbestos fibers and to

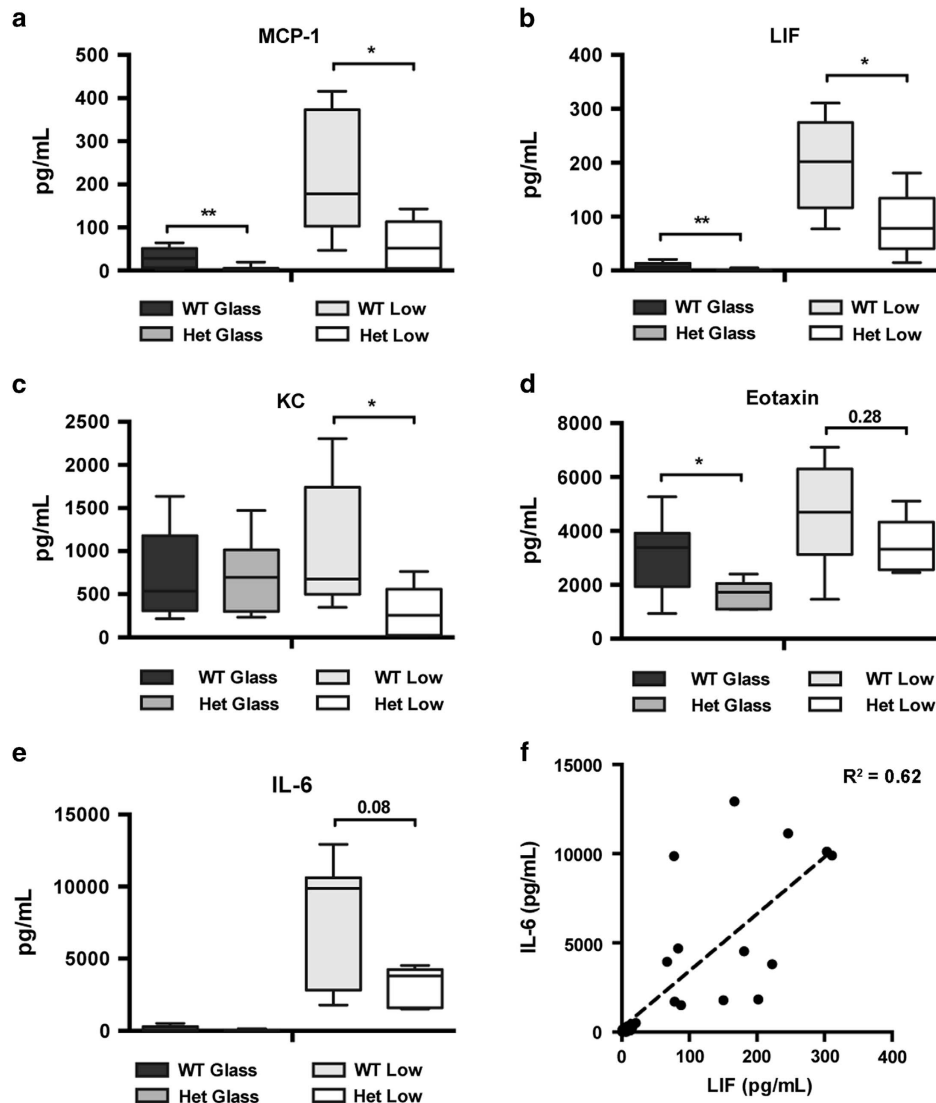


Figure 2. Several cytokines and chemokines are differentially expressed in lavage from *BAP1*^{+/-} mice. The supernatants recovered from the peritoneal lavages were concentrated 45–60 times using Amicon Ultra Centrifuge Filters (EMD Millipore Corporation, Billerica, MA, USA) with a 3000 Dalton cutoff. Levels of 32 cytokines and chemokines were detected in concentrated lavages using human cytokine multiplex kits (EMD Millipore Corporation). Levels of MCP-1 (a), leukemia inhibitory factor (LIF) (b), keratinocyte-derived chemokine (KC) (c), eotaxin (d) and IL-6 (e) in lavages from *BAP1* wild-type and heterozygous mice after short-term exposure to glass beads or crocidolite fibers. Comparisons between heterozygous and wild-type groups were calculated using Mann–Whitney U test for rank comparisons. * $P < 0.05$, ** $P < 0.01$. (f) Correlation of IL-6 and LIF levels (both belonging to the IL-6 family of cytokines) calculated using linear regression. The experiment was replicated two times.

an increased risk of MM following exposure to minimal amounts of asbestos that rarely cause MM in wild-type animals. *BAP1* is a nuclear deubiquitinating enzyme and an important epigenetic regulator via deubiquitination of histone H2A.³¹ Originally discovered in 1998,³² it has several cell-intrinsic tumor-suppressive functions, such as regulation of gene transcription,³³ cell cycle and replication,^{34–36} and DNA damage response.^{37,38} *BAP1* knockdown in MM cell lines has been paradoxically associated to a decreased proliferation, with an accumulation of cells in the S phase of the cell cycle,²² suggesting that *BAP1* loss might promote tumorigenesis inducing a delayed, but more permissive, G1/S checkpoint.²² Heterozygous germline mutations of other important tumor-suppressor genes, such as *BRCA1*, *CDKN2A* and *RB1*, increase risk of cancer specifically to one or very few anatomical sites.³⁹ One of the few tumor-suppressor genes whose germline heterozygosity, similar to *BAP1*, is associated to increased risk of cancer to several sites is *TP53*,

which encodes p53.³⁹ Besides its well-known intrinsic functions, recently a novel non-cell-autonomous tumor-suppressor effect of p53 has been described, via regulation of macrophage polarization and cytokine release.⁴⁰ Our results suggest that germline *BAP1* heterozygosity, similarly to *TP53*, influences *in vivo* macrophage polarization and cytokine release. Indeed, *BAP1*^{+/-} mice exposed to asbestos had significantly more M2-like pro-tumoral macrophages. Also, the chemokines MCP-1 and keratinocyte-derived chemokine, and two cytokines of the IL-6 family (IL-6 itself and leukemia inhibitory factor) are soluble mediators significantly reduced in *BAP1*^{+/-} mice exposed to asbestos. MCP-1 and IL-6 have been reported to increase following asbestos exposure and have been linked to asbestos pathogenesis.^{41,42} Our results support these findings and also suggest that this inflammatory response might be associated with increased immunosurveillance, because lower levels of these and other inflammatory mediators in *BAP1*^{+/-} mice are associated with higher M2/M1 macrophage

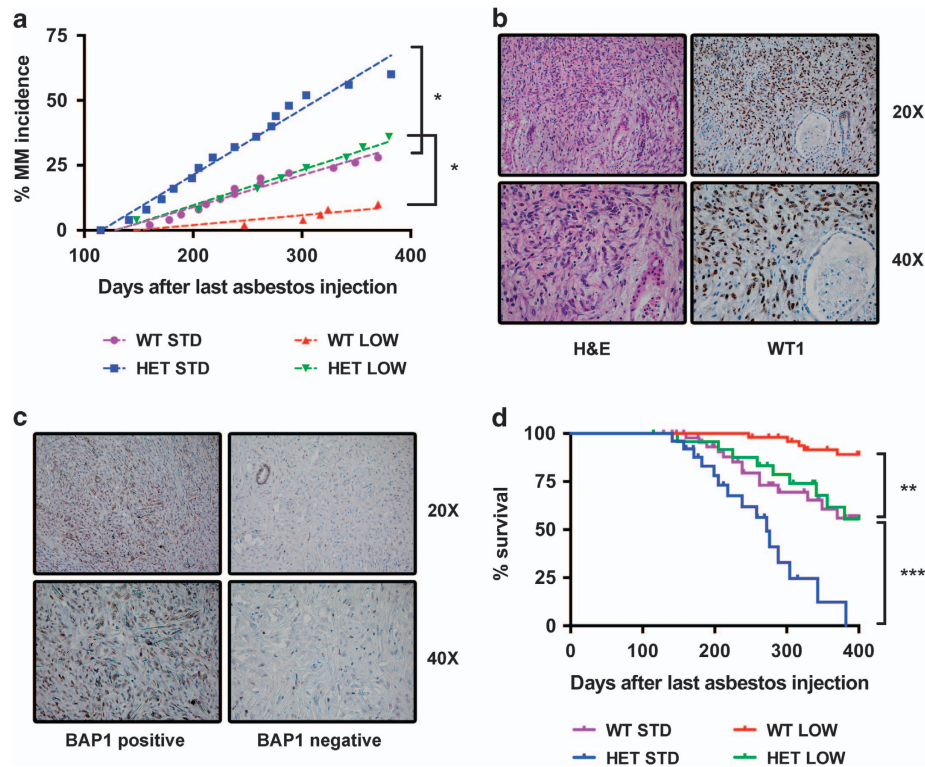


Figure 3. BAP1^{+/-} mice develop more MMs and have shorter survival compared with wild-type littermates. Briefly, BAP1^{+/-} mice ($n = 50$ per group) and BAP1^{+/+} mice ($n = 25$ per group) were injected intraperitoneally every week for 10 weeks with 0.05 mg (low dose) or 0.5 mg (standard dose) of UICC crocidolite; 0.5 mg of glass beads were injected at the same schedule as control. Sample size was estimated to detect a difference in MM incidence between the low-exposed groups $\geq 25\%$. Mice were monitored daily for clinical evidence of abdominal swelling, and killed in presence of respiratory distress, gait instability, unresponsiveness to pain stimuli or when tumor burden was obvious. Upon detection of illness, mice were killed by CO₂ asphyxiation, and all the major organs were evaluated histologically. **(a)** MM incidence in BAP1^{+/-} mice and wild-type littermates after long-term exposure to glass beads or asbestos fibers (standard and low dose) was compared using Fisher's exact test. $*P < 0.05$. **(b)** Formalin-fixed/paraffin-embedded samples were cut into 5- μ m sections and stained with hematoxylin and eosin (H&E) according to the standard procedure. The pathological diagnosis of mesothelioma was based on H&E staining and supported by WT1 nuclear staining in tumor cells. H&E and immunostainings were blindly interpreted by MC and AP, both US board specialized pathologists with expertise in human and animal mesotheliomas.^{14,44,45} **(c)** Tumors were also stained with a rabbit polyclonal anti-BAP1 antibody to evaluate presence and localization of BAP1. **(d)** Survival curves of BAP1^{+/-} mice and wild-type littermates after long-term exposure to asbestos fibers (standard and low dose) were compared using log-rank (Mantel-Cox) test. $**P < 0.01$, $***P < 0.001$. The experiment was performed one time.

ratio and higher MM incidence following asbestos exposure. Interestingly, BAP1 has been recently shown to regulate the myeloid stem cell compartment via complex alterations of the transcriptional profile, possibly via its interaction with transcriptional co-regulators such as Host Cell Factor-1 (HCF-1) and Additional Sex Combs Like-1 (ASXL1).¹⁹

Altogether, our results suggest a novel, complex model of asbestos-induced MM pathogenesis, in which the chronic inflammatory response can have preferentially anti-tumoral or pro-tumoral roles, depending on the cellular and soluble mediators involved. To explain the observed intra- and inter-familial variability of cancer types in germline BAP1-mutated carriers, we hypothesized that MM might be more prevalent in individuals/families exposed to low levels of asbestos,¹⁵ levels that are not, or only marginally, carcinogenic for the population at large. Our results support our hypothesis, as we found that 36% of BAP1^{+/-} mice exposed to low doses of asbestos developed MM, compared with 10% of wild-type mice. Moreover, we found that MM is significantly more frequent in BAP1^{+/-} mice exposed to standard doses of asbestos. This finding corroborates the recent results of Xu *et al.*²⁹ that were obtained in an independent murine model. Both studies found a shorter life span of asbestos exposed BAP1 heterozygous mice compared with wild-type littermates,

suggesting that BAP1^{+/-} mice might develop MM at an earlier age compared with wild-type littermates. Similarly, individuals carrying germline BAP1 mutations are diagnosed with MM at a much younger age compared with sporadic MM cases (mean age 55 years vs 72 years, respectively).¹⁶ Accordingly, although MMs in carriers of germline BAP1 mutations are less aggressive and are associated with survivals from diagnosis of 5–10 years,¹⁶ compared with an average of 1 year in sporadic MM patients, the former die at an earlier age compared with the latter. Survival from diagnosis could not be evaluated in our model, as per IACUC requirements, mice were killed at the first clinical evidences of disease.

Mechanistically, Xu *et al.*²⁹ suggest that the increased MM incidence in BAP1 heterozygous mice was partially related to BAP1-dependent transcriptional regulation of the tumor suppressor retinoblastoma protein. Our findings expand what was previously reported by implicating novel tumor-suppressor effects of BAP1 mediated via the microenvironment.

Moreover, we discovered that BAP1^{+/-} mice exposed to low doses of asbestos developed MMs at a similar rate as BAP1^{+/+} mice exposed to 10 times higher doses. Therefore, although it is not possible to directly compare the low-dose exposure in mice to indoor and/or outdoor environmental exposure in humans, our

findings support our hypothesis that germline *BAP1* heterozygosity increases susceptibility to the carcinogenic effects of low doses of asbestos.

On the basis of our results, we suggest that prevention programs of MM in individuals carrying germline *BAP1* mutations should focus on reducing exposure to even minimal sources of carcinogenic fibers, levels that are within the acceptable 'safe' limits for the population at large (0.1 fibers/cc of air as an 8-hour time-weighted average, as per US Occupational Safety & Health Administration standards⁴³). Finally, although our model focuses on asbestos as a trigger, this novel non-cell-autonomous tumor-suppressive function of *BAP1* may not be restricted to the peritoneal compartment or to the asbestos stimulation, and may contribute to the large numbers and diverse types of tumors that arise in carriers of the *BAP1* cancer syndrome.

CONFLICT OF INTEREST

M Carbone has pending patent applications on *BAP1* and provides consultation for mesothelioma expertise and diagnosis. The remaining authors declare no conflicts of interests.

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- 43 Occupational Safety & Health Administration. *OSHA FactSheet: Asbestos*, 2014. <http://www.osha.gov/Publications/OSHA3507.pdf>.
- 44 Kroczyńska B, Cutrone R, Bocchetta M, Yang H, Elmishad AG, Vacek P *et al*. Crocidolite asbestos and SV40 are cocarcinogens in human mesothelial cells and in causing mesothelioma in hamsters. *Proc Natl Acad Sci USA* 2006; **103**: 14128–14133.
- 45 Comertpay S, Pastorino S, Tanji M, Mezzapelle R, Strianese O, Napolitano A *et al*. Evaluation of clonal origin of malignant mesothelioma. *J Transl Med* 2014; **12**: 301.
- 46 Qi F, Okimoto G, Jube S, Napolitano A, Pass HI, Laczko R *et al*. Continuous exposure to chrysotile asbestos can cause transformation of human mesothelial cells via HMGB1 and TNF-alpha signaling. *Am J Pathol* 2013; **183**: 1654–1666.

Supplementary Information accompanies this paper on the Oncogene website (<http://www.nature.com/onc>)

University of Hawaii at Manoa
CURRICULUM VITAE

Haining Yang



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University of Hawaii Cancer Center

Current Address:

Date of Birth:

EDUCATION:

Dates	Institution & location	Degrees or status	Subject
1992-1997	Shandong Medical University, China	M.D.	Medicine
1997-2002	Shandong University, China	Ph.D.	Biology
2003-2006	Loyola University, Chicago, IL	Postdoctoral Fellow	Cancer Biology

POSITIONS:

2002-2003	Project Manager, New England Biolabs, P. R. China
2006-2007	Junior Researcher, Cancer Research Center of Hawaii, University of Hawaii at Manoa
2007-2009	Assistant Researcher (non-tenure track), Cancer Research Center of Hawaii, University of Hawaii at Manoa
2009-2013	Assistant Researcher, Full Member, University of Hawaii Cancer Center, University of Hawaii at Manoa
2009-2013	Assistant Professor (tenure track), John A. Burns School of Medicine, University of Hawaii at Manoa
2013-2014	Associate Professor (tenure track), University of Hawaii Cancer Center, University of Hawaii at Manoa, Honolulu, USA
2014-Present	Associate Professor (tenured), University of Hawaii Cancer Center, University of Hawaii at Manoa, Honolulu, USA

HONORS AND AWARDS:

2005	EU Marie Curie Scholarship by the European Commission Marie Curie Actions Program
2008	AACR Landon Innovator Award for International Collaboration in Cancer Research

PEER-REVIEWED PUBLICATIONS:

1. Yang H, Yu X, et al. Cloning and In Vitro Expression of HPV58E6 Gene. Acta Academiae Medicinae Shandong, 2001, 39(6): 488-494.
2. Yang H, Yu X, et al. Study On the Interaction of Bovine Papillomavirus E6 Protein with p73. Chin J Microbiol Immunol, 2001, 21(4): 363.
3. Yang H, Yu X, et al. Interaction of Bovine Papillomavirus E6 Protein with p73. Basic Medical Sciences and Clinics, 2002, 22(2): 138-142.
4. Yang H, Yu X, et al. The Human Papillomavirus type 58 E6 promotes the degradation of the tumor suppressor p53. Basic Medical Sciences and Clinics, 2002, 22(4): 346-350.
5. Yang H, Yu X, et al. Study on the interaction of human papillomavirus type58 E6 with p53 protein. Chin J Microbiol Immunol, 2003, 23(4): 296-299.
6. Kroczyńska B, Cutrone R, Bocchetta M, Yang H, Elmishad AG, Vacek P, Ramos-Nino M, Mossman BT, Pass

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7. Yang H, Bocchetta M, et al. TNF- α inhibits asbestos induced cytotoxicity via a NF- κ B dependent pathway, a possible mechanism for asbestos-induced oncogenesis. *Proc Natl Acad Sci USA*, 2006, 103(27): 10397- 10402. PMID: 16798876. PMCID: PMC1502469.
 8. Carbone M, Strianese O, Theos K and Yang H. Mesothelioma. *Hawaii'i Medical Journal*, 2007, 66(2): 48-50. PMID: 17393919.
 9. Beck AK, Pass HI, Carbone M and Yang H. Ranpirnase as a potential anti-tumor ribonuclease treatment for mesothelioma and other malignancies. *Future Oncology*, 2008, 4(3): 341-349. PMID: 18518759.
 10. Rivera Z, Strianese O, Bertino P, Yang H, Pass H, Carbone M. The relationship between simian virus 40 and mesothelioma. *Current Opinion in Pulmonary Medicine*, 2008, 14(4): 316-321. PMID: 18520265.
 11. Yang H, Testa JR and Carbone M. Mesothelioma Epidemiology, Carcinogenesis and Pathogenesis. *Current Treatment Options in Oncology*. 2008, 9(2-3): 147-157. PMID: 18709470. PMCID: PMC2717086.
 12. Yang H, Nasu M, Strianese O, Rivera Z, Luecke L, Carbone M. The legacy of "miracle mineral": asbestos and cancer. *Hawaii Med J*. 2009; 68(1): 18-20. PMID: 19365925.
 13. Yang H, Rivera Z, Jube S, Nasu M, Bertino P, Goparaju C, Franzoso G, Lotze MT, Krausz T, Pass HI, Bianchi ME, Carbone M. Programmed necrosis induced by asbestos in human mesothelial cells causes high-mobility group box 1 protein release and resultant inflammation. *Proc Natl Acad Sci USA*, 2010, 107(28): 12611-12616. PMID: 20616036. PMCID: PMC2906549.
 14. Goparaju CM, Blasberg JD, Volinia S, Palatini J, Ivanov S, Donington JS, Croce C, Carbone M, Yang H and Pass HI. Onconase mediated NF κ B downregulation in malignant pleural mesothelioma. *Oncogene*, 2011, 30(24): 2767-2777. PMID: 21317924. PMCID: PMC3118086.
 15. Zhang L, Qi F, Gaudino G, Strianese O, Yang H, Morris P, Pass HI, Nerurkar VR, Bocchetta M, Carbone M. Tissue tropism of SV40 transformation of human cells: role of the viral regulatory region and of cellular oncogenes. *Genes and Cancer*, 2010, 1(10): 1008-1020. PMID: 21779427. PMCID: PMC3092263.
 16. Qi F, Carbone M, Yang H, Gaudino G. Simian virus 40 transformation, malignant mesothelioma and brain tumors. *Expert Rev Respir Med*. 2011, 5(5): 683-697. PMID: 21955238. PMCID: PMC3241931.
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 18. Nasu M, Carbone M, Gaudino G, Ly BH, Bertino P, Shimizu D, Morris P, Pass HI, Yang H. Ranpirnase interferes with NF- κ B pathway and MMP9 activity, inhibiting Malignant Mesothelioma cell invasiveness and xenografts growth. *Genes Cancer*. 2011, 2(5): 576-584. PMID: 21901170. PMCID: PMC3161417.
 19. Testa JR, Cheung M, Pei J, Below JE, Tan Y, Sementino E, Cox NJ, Dogan AU, Pass HI, Trusa S, Hesdorffer M, Nasu M, Powers A, Rivera Z, Comertpay S, Tanji M, Gaudino G, Yang H, Carbone M. Germline Bap1 mutations predispose to malignant mesothelioma. *Nat Genet*. 2011, 43(10): 1022-1025. PMID: 21874000. PMCID: PMC3184199.
 20. Carbone M, Ly BH, Dodson RF, Pagano I, Morris PT, Dogan UA, Gazdar AF, Pass HI, Yang H. Malignant Mesothelioma: Facts, Myths and Hypotheses. *Journal of Cellular Physiology*, 2012, 227(1): 44-58. PMID: 21412769. PMCID: PMC3143206.
 21. Carbone M and Yang H. Molecular pathways: targeting mechanisms of asbestos and erionite carcinogenesis in mesothelioma. *Clin Cancer Res*. 2012, 18(3): 598-604. PMID: 22065079. PMCID: PMC3291331.
 22. Jube S, Rivera Z, Bianchi ME, Powers A, Wang E, Pagano IS, Pass HI, Gaudino G, Carbone M, Yang H. Cancer Cell secretion of the DAMP protein HMGB1 supports progression in malignant mesothelioma. *Cancer Res*. 2012, 72(13): 3290-3301. PMID: 22552293. PMCID: PMC3389268. (This paper made the cover of the July 1st 2012 issue of *Cancer Res*.)
 23. Rivera Z, Ferrone S, Wang X, Jube S, Yang H, Pass HI, Kanodia S, Gaudino G, Carbone M. CSPG4 as a Target of Antibody-Based Immunotherapy for Malignant Mesothelioma. *Clin Cancer Res*. 2012, 18(19): 5352-5363. PMID: 22893632. PMCID: PMC3463742.
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25. Croce A, Musa M, Allegrina M, Rinaudo C, Baris YI, Dogan AU, Powers A, Rivera Z, Bertino P, Yang H, Gaudino G, Carbone M. Micro-Raman spectroscopy identifies crocidolite and erionite fibers in tissue sections. *J. Raman Spectrosc.* 2013, 44: 1440-1445.
 26. Bertino P, Panigada M, Soprana E, Bianchi V, Bertilaccio S, Sanvito F, Rose AH, Yang H, Gaudino G, Hoffmann PR, Siccardi A, Carbone M. Fowlpox-based survivin vaccination for malignant mesothelioma therapy. *Int J Cancer.* 2013, 133(3): 612-623. PMID: 23335100. PMCID: PMC3663911.
 27. Carbone M, Yang H, Pass HI, Krausz T, Testa JR, Gaudino G. BAP1 and Cancer. *Nat Rev Cancer.* 2013, 13(3): 153-159. PMID: 23550303. PMCID: PMC3792854.
 28. Ferris L. K., Kupetsky E. A., Carbone M., & Yang H. Could testing for BAP1 germline mutations be a useful tool for early melanoma diagnosis? *Expert Review of Dermatology.* 2013, 8(2): 107-109.
 29. Qi F, Okimoto G, Jube S, Napolitano A, Pass HI, Laczko R, DeMay RM, Khan G, Tiirikainen M, Rinaudo C, Croce A, Yang H, Gaudino G and Carbone M. Continuous Exposure to Chrysotile Asbestos Can Cause Transformation of Human Mesothelial Cells via HMGB1 and TNF- α Signaling. *Am J Pathol.* 2013, 183(5): 1654-1666. PMID: 24160326.
 30. Gualtieri AF, Giacobbe C, Rinaudo C, Croce A, Allegrina M, Gaudino G, Yang H, Carbone M. Preliminary results of the spectroscopic and structural characterization of mesothelioma inducing crocidolite fibers injected in mice. *Periodico di Mineralogia*, 82:299-312, 2013.
 31. Comertpay S, Pastorino S, Tanji M, Mezzapelle R, Strianese O, Napolitano A, Baumann F, Weigel T, Friedberg J, Sugarbaker P, Krausz T, Wang E, Powers A, Gaudino G, Kanodia S, Pass HI, Parsons BL, Yang H, Carbone M. Evaluation of clonal origin of malignant mesothelioma. *J Transl Med.* 2014. Dec 4;12:301. PMID: 25471750
 32. Cioce M, Canino C, Goparaju C, Yang H, Carbone M, Pass HI. Autocrine CSF-1R signaling drives mesothelioma chemoresistance via AKT activation. *Cell Death Dis*, 2014 Apr 10; 5:e1167, doi: 10.1038/cddis.2014.136.
 33. Tekade R, Youngren-Ortiz S, Yang H, Haware R, Chougule M. Designing Hybrid Onconase Nanocarriers for Mesothelioma Therapy: A Taguchi Orthogonal Array and Multivariate Component Driven Analysis. *Mol Pharm.* 2014 Oct 6;11(10):3671-83.
 34. Baumann F, Flores E, Napolitano A, Kanodia S, Taioli E, Pass H, Yang H, Carbone M. Mesothelioma patients with germline BAP1 mutations have 7-fold improved long-term survival. *Carcinogenesis.* 2015 Jan;36(1):76-81. PMID: 25380601
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 36. Carbone M, Gaudino G, Yang H. Recent insights emerging from malignant mesothelioma genome sequencing. *J Thorac Oncol.* 2015 Mar;10(3):409-11. PMID: 25695218
 37. Yang H, Pelegrini L, Napolitano A, Giorgi C, Jube S, Preti A, Jennings CJ, De Marchis F, Flores EG, Larson D, Pagano I, Tanji M, Powers A, Kanodia S, Gaudino G, Pastorino S, Pass HI, Pinton P, Bianchi ME and Carbone M. Aspirin delays mesothelioma growth by inhibiting HMGB1-mediated tumor progression. *Cell Death & Disease.* 2015 Jun 11. PMID: 26068794
 38. Napolitano A, Pelegrini L, Anwasha D, Larson D, Tanji M, Flores EG, Kendrick B, Lapid D, Powers A, Kanodia S, Pastorino S, Pass HI, Dixit V, Yang H and Carbone M. Minimal asbestos exposure in germline BAP1 heterozygous mice is associated with deregulated inflammatory response and increased risk of mesothelioma. *Oncogene.* 2015 Jun 29. [Epub ahead of print]. PMID: 26119930
 39. Bononi A, Napolitano A, Pass HI, Yang H, Carbone M. Latest developments in our understanding of the pathogenesis of mesothelioma and the design of targeted therapies. *Expert Rev Respir Med.* 2015 Oct;9(5):633-54. PMID: 26308799
 40. Carbone M, Flores EG, Emi M, Johnson TA, Tsunoda T, Behner D, Hoffman H, Hesdorffer M, Nasu M, Napolitano A, Powers A, Minaai M, Baumann F, Bryant-Greenwood P, Lauk O, Kirschner MB, Weder W, Opitz I, Pass HI, Gaudino G, Pastorino S and Yang H. Combined Genetic and Genealogic Studies Uncover a Large BAP1 Cancer Syndrome Kindred, Tracing Back Nine Generations to a Common Ancestor from the 1700s. *PLoS Genet.* 2015 Dec 18;11(12):e1005633. doi: 10.1371/journal.pgen.1005633. PMID: 26683624
 41. Napolitano A, Antoine DJ, Pellegrini L, Baumann F, Pagano I, Pastorino S, Goparaju CM, Prokrym K, Canino C, Pass HI, Carbone M, Yang H. HMGB1 and its hyper-acetylated isoform are sensitive and specific serum biomarkers to detect asbestos exposure and to identify mesothelioma patients. *Clin Cancer Res.* Published

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43. Larson D, Powers A, Ambrosi JP, Tanjji M, Napolitano A, Flores EG, Baumann F, Pellegrini L, Jennings CJ, Buck BJ, McLaurin BT, Merkle D, Robinson C, Morris P, Dogan M, Dogan AU, Pass HI, Pastorino S, Carbone M, Yang H. Investigation palygorskite's role in the development of mesothelioma in southern Nevada: Insights into fiber-induced carcinogenicity. J Tox and Envir Health Part B. 2016 (accepted)

BOOK CHAPTERS:

1. Yang H, Pass H. Blood-based Biomarkers of Mesothelioma. In: Malignant Pleural Mesothelioma: Science and Clinical Practice. Section on Clinical Presentation & Diagnosis. Michele Carbone and Shreya Kanodia (Ed). (submitted)
2. Yang H, Gaudino G. Mechanisms of Mesothelioma Initiation and Progression. In: Malignant Pleural Mesothelioma: Science and Clinical Practice. Section on Clinical Presentation & Diagnosis. Michele Carbone and Shreya Kanodia (Ed). (submitted)
3. Napolitano A, Pellegrini L, Yang H, Carbone M. BAP1 mutations in malignant mesothelioma. Malignant pleural mesothelioma: current status and future direction. Tommaso Claudio Mineo (Ed). (submitted)
4. Napolitano A, Jube S, Gaudino G, Pass HI, Carbone M and Yang H. Asbestos-induced chronic inflammation and cancer. In: Cancer and Inflammation Mechanisms: Chemical, Biological, and Clinical Aspects. Shosuke Kawanishi, Hiroshi Ohshima and Yusuke Hiraku (Ed.) pp 223-234. 2014
5. Carbone M and Yang H. SV40. Encyclopedia of Cancer, 2nd Ed., Schwab M (Ed.). Springer. (In press).
6. Yang H, Pass H and Carbone M. Pathogenesis of mesothelioma. In: Mesothelioma from Bench Side to Clinic. Baldi A. (ed.), (Nova Biomedical, New York) pp 105-116. 2008

PROFESSIONAL ACTIVITY:

Scientific Advisory board (SAB) of Mesothelioma Applied Research Foundation (MARF).

PEER REVIEW ACTIVITIES:

1. Manuscript review:

Served as a member of Editorial Board for the following journals:

Frontiers Oncology
Frontiers in Cell and Developmental Biology

Served as a peer reviewer for the following journals:

American Journal of Respiratory Cell and Molecular Biology
BMC Cancer
Cancer
Cancer Research
Clinical Cancer Research
Current Cancer Therapy Reviews
Free Radical Biology and Medicine
Gene Therapy & Molecular Biology
Lung Cancer
Journal of Cellular and Molecular Medicine
Journal of Hazardous Materials
PLOS ONE
Stem Cell
Translational Research

2. Grant Review:

NIEHS/NIH Career Development Award (K08/K23)
Visionary Postdoctoral Fellowship Award (VPF-1), Department of Defense (DoD) Congressionally Directed Medical Research Programs (CDMRP) Peer reviewed Cancer Research Program (PRCRP)
Asbestos-related Disease Project Grant Award, British Lung Foundation.
Army Research Office FY12 In-House Laboratory Independent Research (ILIR) Research Proposal
Chateaubriand Fellowship, Office of Science and Technology of the U.S. Embassy of France
Mesothelioma Applied Research Foundation Grant

3. Additional Reviewer activity:

Serve as a scientist reviewer for the mode of action (MOA) meeting on asbestos and related mineral fiber, sponsored by the National Institute of Environmental Health Sciences (NIEHS) and the US Environmental Protection Agency (USEPA)
Review of the NIOSH Research Roadmap on Asbestos Fibers and Other Elongated Mineral Particles

MEMBERSHIP IN SOCIETIES/ORGANIZATIONS:

2005-present American Association for Cancer Research
2010-present Society for Immunotherapy of Cancer
2011-present The Geological Society of America
2015-present American Society for Cell Biology

INTERNATIONAL MEETING ORGANIZATION ACTIVITIES:

2011 Third Annual Translational Cancer Medicine Symposium in Hawaii, Scientific & Organizing Committee
2012 4th International HMGB1 workshop and Translational Cancer Medicine Symposium in Hawaii, Scientific & Organizing Committee
2015 7th International Symposium of DAMP and HMGB1. Bonn, Germany. September 2015. Organizing Committee
2015 International Conference on Mesothelioma in Populations Exposed to Naturally Occurring Asbestiform Fibers. Honolulu, US. November 2015. Organizing Committee

UNIVERSITY OF HAWAII SERVICE:

2010 - 2011 Junior Faculty Representative, Executive Committee, University of Hawaii Cancer Center
2009 - present Director, Basic Science & Translational Research Seminar Series and Thoracic Oncology Seminar Series
2009 - present Committee on Bioinformatic Shared Resource, University of Hawaii Cancer Center
2011- present Committee on Microscopy and Imaging Shared Resource, University Hawaii Cancer Center
2012 - 2014 Search Committee on Basic Science faculty recruitment, University of Hawaii Cancer Center
2014 First speaker in the professional staff development initiative program for the Financial Management officer (FMO) at University of Hawaii.
2015 Served on the Departmental Personnel Committee of University of Hawaii Cancer Center to review applications for tenure/promotion

PUBLIC/COMMUNITY SERVICE:

2008- Representative volunteer, ACS Relay for Life Walk and Activities
2011 Representative volunteer of UH Cancer Center, Bank of Hawaii Employee Giving Expo Event
2011 Invited speaker for the Medical Day in China Town, Honolulu, HI
2011 Invited speaker for the Scientific Teachers' Educational visit of the UH Cancer Center
2013 Invited speaker for the Medical Day event, Honolulu, HI
2014 Invited speaker for the "Science Café" sponsored by Hawaii Academy of Science.

TEACHING:

1. Course: Molecular Biology of Cancer – CMB 654C 001

2. List of fellows that I participated in training.

1) Postdoctoral fellow training:

Angela Bononi
David Larson
Ekaterina Kashkina
Erin Flores
Laura Pellegrini
Masaki Nasu
Sandro Jube
Vishal Singh Negi

2) Research assistant fellow training:

Agata Szymiczek
Dusty Behner
Elia Bruno
Ronghui Xu
Shuangjing Li
Sylvia Koo
Xiaohua Wu
Yasutaka Takinishi

3). Graduate student mentoring:

Andrea Napolitano (PhD candidate)
Amrish Sharma (PhD candidate, graduated in 2011)
Cormac Jennings (PhD candidate, graduated in 2012)
Fang Qi (PhD candidate, graduated in 2013)
Jiaming Xue (PhD candidate)
Kimberly Theos (Master student, graduated in 2009)
Lauren Fonseca (PhD candidate, graduated in 2014)
Lauren Gardner (Master student, graduated in 2013)
Laura Pellegrini (PhD candidate, graduated in 2013)
Masaki Nasu (PhD candidate, graduated in 2012)
Mika Tanji (Master student, graduated in 2012)
Oriana Strianese (PhD candidate, graduated in 2009)
Rosanna Mezzapelle (PhD candidate, graduated in 2013)
Sabahattin Comertpay (PhD candidate, graduated in 2012)
Zeyana S. Rivera (PhD candidate, graduated in 2012)

4). Medical student research work mentoring:

Stephen Chun
Sara Harris

5). Pathology resident research work mentoring:

Lei Zhang, MD, PhD.

6). Summer student training:

Andrew Kuriyama
Brandon Taylor
Jennifer Teruya

Hauoli Gardner
Michael Minaai
Michelle Tagorda
Olivia Manayan
Wai Yan Lam
Sarah Hirata
Katharine Wong
Lahaina Zoller
Jamie Takayesu
Liam Arnade-Colwill

ABSTRACTS AND MEETING PRESENTATION:

1. Yang H, Bocchetta M, et al. 13th Euroconference on Apoptosis, 2005, Oct 1-5, Budapest. (Poster)
2. Yang H, Cutrone R, et al. Experimental Biology Conference, 2005, LB385: 84. (Poster)
3. Kroczyńska B, Cutrone R, Bocchetta M, Yang H, et al. American Association for Cancer Research Meeting, 2005, April 16-20, Anaheim, California. (Poster)
4. Yang H, Bocchetta M, et al. TNF- α inhibits asbestos induced cytotoxicity via a NF- κ B dependent pathway. IMIG meeting in Chicago, Oct 18-22 2006. Lung Cancer, 2006, 54(1): S21. (Oral presentation)
5. Kroczyńska B, Cutrone R, Bocchetta M, Yang H, et al. IMIG meeting, Oct 18-22 2006, Chicago. (Poster discussion)
6. Carbone M, Yang H, Pass H. Early Detection of Mesothelioma in the Mesothelioma Villages in Turkey. Gordon Conference in Ventura, CA, January 21-26, 2007. (Oral presentation and poster)
7. Yang H, Bocchetta M et al. TNF- α plays a critical role in asbestos-induced oncogenesis. American Association for Cancer Research Meeting, 2007, April, Los Angeles. (Poster)
8. Carbone M, Pass HI, Yang H, et al. Early Detection of Mesothelioma. American Association for Cancer Research Meeting, 2007, April, Los Angeles. (Poster)
9. Kroczyńska B, Yang H and Carbone M. Co-carcinogenesis study of wild type SV40 virus and crocidolite asbestos. American Association for Cancer Research Meeting, 2008, April, San Diego. (Poster)
10. Yang H, Carbone M. Onconase inhibits mesothelioma cells invasion induced by TNF- α . International Mesothelioma Interest Group (IMIG) meeting, 2008, September, Amsterdam. (Oral presentation)
11. Bocchetta M, Yang H, Elias S, et al. The SV40 large T antigen-p53 complexes bind and activate the IGF-1 promoter stimulating cell growth. International Mesothelioma Interest Group (IMIG) meeting, 2008, September, Amsterdam. (Poster discussion)
12. Yang H, Nasu M et al. Asbestos-induced Human Mesothelial Cell Death causes the Release of HMGB1 and Resultant Inflammation. The Third International Damage Associated Molecular Pattern Molecules (DAMPs) and Alarmins Symposium, 2008, September, Pittsburgh. (Poster)
13. Yang H, Nasu M et al. Asbestos-induced HM Death Causes Inflammation. International Society for Biological Therapy of Cancer (ISBC) 23rd Annual Meeting, 2008, October, San Diego. (Oral Presentation)
14. Nasu Masaki, Carbone M and Yang H. Studies of mesothelioma cells invasion induced by TNF- α and the inhibition effect of Onconase. AACR special meeting "Chemical and Biological Aspects of Inflammation and Cancer". 2008, October, Oahu. (Poster)
15. Yang H. Mechanism of Asbestos Carcinogenesis: Possible New Targets for Cancer Prevention. Translational Cancer Medicine Symposium, 2009, February, Hawaii. (Oral Presentation)
16. Bocchetta M, Yang H, Carbone M. P53-SV40 Tag complexes have oncogenic activity because they activate the IGF-1 signaling pathway and promote cell growth. American Association for Cancer Research 100th Annual Meeting, 2009, April, Denver, Colorado. (Poster)
17. Carbone M, Yang H. Early Detection of Mesothelioma in Cappadocia. 6th Early Detection Research Network (EDRN) Scientific Workshop, 19th EDRN Steering Committee Meeting, 2009, September, Bethesda. (Oral presentation)
18. Yang H, Pass HI, Carbone M. Role of SMRP and Osteopontin for Early Detection of Human Malignant Mesothelioma. NCI translational Science Meeting, 2009, November, Vienna. (Poster)
19. Rivera Z, Ferrone S, Yang H and Carbone M. Inhibition of in vitro growth of human mesothelioma cells by CSPG4-specific monoclonal antibodies. American Association for Cancer Research 101th Annual Meeting,

- 2010, April, Washington D.C. (Poster)
20. Jube S, Rivera Z, Gaudino G, Pass HI, Bianchi ME, Carbone M, Yang H. HMGB1, a potential new target for mesothelioma therapy. American Association for Cancer Research 102nd Annual Meeting, 2011, April, Washington D.C. (Poster)
 21. Yang H, Carbone M. HMGB1 mediates mineral fibers carcinogenesis. Geological Society of America Annual Meeting, 2011, October, Minneapolis, MN (Oral Presentation)
 22. Carbone M, Yang H. Erionite exposure in N. Dakota and in the Turkish villages with mesothelioma. Geological Society of America Annual Meeting, 2011, October, Minneapolis, MN (Poster)
 23. Yang H, Jube S, Gaudino G, Pass HI, Bianchi ME, Carbone M. HMGB1 and Mesothelioma. 3rd HMGB1 workshop, 2011, October, Pittsburgh, PA (Poster)
 24. Carbone M, Ferris LK, Baumann F, Napolitano A, Lum CA, Flores EG, Gaudino G, Powers A, Bryant-Greenwood P, Krausz T, Hyjek E, Tate R, Friedberg J, Weigel T, Pass HI, Yang H. The BAP1 Cancer Syndrome. 11th International Conference of the International Mesothelioma Interest Group (iMig). 2012, September, Boston, MA (Oral Presentation)
 25. Yang H, Carbone M. Studies on Prevention and Therapy for mesothelioma, a cancer model of Gene-Environment Interaction. The 7th ICBBE 2013, September, Beijing, China (Oral Presentation)
 26. Qi F, Okimoto G, Jube S, Pass HI, Laczko R, DeMay RM, Khan G, Tiirikainen M, Yang Y, Gaudino G, Carbone M. Common and different effects induced in primary human mesothelial cells and mice exposed to chrysotile or crocidolite asbestos. American Association for Cancer Research 2013 Annual Meeting. Washington, DC, April 6-10, 2013. (Poster)
 27. Carbone M and Yang H. BAP1 mutation in mesothelioma and BAP1 Cancer Syndrome. American Association for Cancer Research 2014 Annual Meeting, San Diego, CA, April 5-9, 2014. (Poster)
 28. Carbone M and Yang H. BAP1 gene mutation, mesothelioma pathogenesis and a novel cancer syndrome. AACR Cancer Susceptibility and Cancer Susceptibility Syndromes conference, San Diego, CA, January 29-February 1, 2014. (Poster)
 29. Carbone M, Nasu M, Tanji M, Pastorino S, Flores E, Baumann F, Powers A, Kanodia K, Gaudino G, Pass, HI, Emi E, Yang H. BAP1 mutations in sporadic mesothelioma. 2014 International Conference of the International Mesothelioma Interest Group (iMig), Cape Town, S. Africa, October 21-24, 2014. (Oral presentation)
 30. Yang H, Pelegri L, Napolitano A, Giorgi C, Jube S, Preti A, Jennings CJ, De Marchis F, Flores EG, Larson D, Pagano I, Tanji M, Powers A, Kanodia S, Gaudino G, Pastorino S, Pass HI, Pinton P, Bianchi ME and Carbone M. Aspirin delays tumor growth by targeting High-Mobility Group Box 1 protein. International Mesothelioma Interest Group (iMig) Conference. Cape Town, South Africa. October 21-24. 2014. (Oral presentation)
 31. Yang H, Pelegri L, Napolitano A, Gaudino G, Pastorino S, Pass HI, and Carbone M. New findings on the mechanisms and potential therapeutic targets for mesothelioma. AACR Tumor Immunology and Immunotherapy: A New Chapter. Orlando, Florida, December 1 - 4, 2014. (Poster)
 32. Yang H, Carbone M. Novel Therapeutic Approaches For Human Malignant Mesothelioma. AACR ECCO meeting. Florence June 20-23, 2015. (Poster)
 33. Pelegri L, Napolitano A, Gaudino G, Pastorino S, Pass HI, Carbone M and Yang H. HMGB1 targeting by Ethyl Pyruvate Suppresses Malignant Phenotype of Human Mesothelioma. ASCB Annual Meeting. San Diego, December 12-16, 2015. (Poster)
 34. Napolitano A, Antoine DJ, Pellegrini L, Baumann F, Pagano I, Pastorino S, Goparaju CM, Prokrym K, Canino C, Pass HI, Carbone M, Yang H. HMGB1 and its hyper-acetylated isoform are sensitive and specific serum biomarkers for asbestos exposure and mesothelioma detection. American Association for Cancer Research 2016 Annual Meeting, New Orleans, LA, April 5-9, 2014. (Poster)

INVITED SPEAKER

1. 1st Translational Cancer Medicine Symposium, 2009, February, Honolulu. "Mechanism of Asbestos Carcinogenesis: Possible New Targets for Cancer Prevention."
2. American Association for Cancer Research (AACR) 2010, April, Washington DC. "New Developments in the Mechanisms of Asbestos Carcinogenesis."
3. International Symposium on Malignant Mesothelioma, 2010, June, Washington DC. "Programmed necrosis induced by asbestos in human mesothelial cells causes HMGB1 release and resultant inflammation".
4. University of Rome La Sapienza, 2011, July, Rome, Italy. "HMGB1 mediates asbestos carcinogenesis".

5. 1st Joint Meeting: Advances in Cancer Research between Magna Grecia University and University of Hawaii. Distinguished Lecture, Istituto Nazionale Tumori "Regina Elena", 2011, July, Catanzaro, Italy. "Studies on Asbestos Carcinogenesis and Mesothelioma Pathogenesis".
6. National Institute for Environmental Health Sciences (NIEHS), workshop on erionite, 2011 October, Research Triangle Park, North Carolina. "Role of HMGB1 in Asbestos and Erionite Carcinogenesis."
7. 3rd Translational Cancer Medicine Symposium, 2011, December, Honolulu. "Potential Targets for Mesothelioma Prevention and Therapy."
8. 5th Annual World Cancer Congress, 2012, May, Beijing, China. "Potential Targets for Mesothelioma Prevention and Therapy." (Also chairing the session of New Targets, Biomarker & New Technologies for Anticancer Therapeutics and Drug Discovery")
9. HMGB1 and Damage associated molecular pattern molecules (DAMPs) ThinkTank meeting, 2012, July, Heidelberg, Germany. "The Role of HMGB1 in Mesothelioma Development and Progression."
10. 11th International Conference of the International Mesothelioma Interest Group (iMig), 2012, September, Boston. "Role of HMGB1 in Asbestos Carcinogenesis and Mesothelioma." (Also chairing the session of "Animal Models for Mesothelioma")
11. 4th International HMGB1 Symposium, 2013, February, Honolulu, Hawaii. "The Role of HMGB1 and DAMPs in Mesothelioma".
12. Mesothelioma Applied Research Foundation 10th International Conference, 2013, March, Las Vegas, NV. "New research findings on mesothelioma overview".
13. Liaocheng People's hospital, 2013, September, Liaocheng, China. "Current studies on mesothelioma: a cancer model of Gene-Environment interaction".
14. HMGB1 World Congress, 2013, October, New York. "HMGB1 drives asbestos carcinogenesis and mesothelioma growth: options for prevention and therapy".
15. International Symposium of Malignant Mesothelioma. 2014, March, Alexandria, VA. "Aspirin targeting HMGB1 serving as a potential strategy for mesothelioma prevention and therapy".
16. Zhejiang Cancer Research Institute. Zhejiang Cancer Hospital. 2014, June, Zhejiang, China. "Current studies on mesothelioma early detection, prevention and therapy."
17. Sidra Medical and Research Center. 2014, October, Doha, Qatar. "Novel targets for mesothelioma prevention and therapy".
18. International Symposium of Malignant Mesothelioma. Mesothelioma Applied Research Foundation. 2015, March, Bethesda, Maryland. "Mesothelioma biomarkers and early detection".
19. University of Ferrara. 2015, April, Ferrara, Italy. "HMGB1, the driving force for mesothelioma development and progression".
20. New Frontiers in Oncology. 2015, April, Rome, Italy. "HMGB1 and the Pathogenesis of Mesothelioma".
21. Mt Sinai Hospital. 2015, June. New York, NY. "Novel biomarker of asbestos exposure and mesothelioma".
22. Magna Graecia University and Tommaso Campanella Cancer Center. 2015, September. Catanzaro, Italy. "HMGB1, a biomarker of asbestos carcinogenesis and potential therapeutic target for mesothelioma"
23. 7th International Symposium DAMPS and HMGB. 2015, September. Bonn, Germany. "HMGB1 as biomarker for asbestos exposure and mesothelioma detection".
24. University of Torino. 2015, November, Turin, Italy. "Mechanisms of Asbestos Carcinogenesis and HMGB1"
25. 13th International Conference of the International Mesothelioma Interest Group (iMig), 2016, May, Birmingham, UK. "Mechanisms of asbestos carcinogenesis and HMGB1".

PATENTS:

Biomarker of Asbestos Exposure and Mesothelioma

Inventors: Yang, H., Carbone, M., Pass, H. I.

Patent No.: US 9,244,074 B2

Date of Patent: Jan. 26, 2016

Abstract: Methods of diagnosing asbestos exposure or mesothelioma, and methods of differentiating whether a tumor of the lung is lung cancer or mesothelioma.

Methods and Kits for Analysis of HMGB1 Isoforms

Inventors: Yang, H., Carbone, M.

Filing #: US Provisional Patent application no. 62/106,092

Year Filed: 2015

Abstract: Methods of determining signatures of HMGB1 isoforms in a subject, and the use of HMGB1 and its isoforms as biomarkers for asbestos exposure and mesothelioma detection.

Treatment and Prevention of Cancer with HMBG1 Antagonists

Inventors: Yang, H., Carbone, M., Bianchi, M.E.

Filing #: US Application no. 14/123,607

Year Filed: 2013

Abstract: Methods and Compositions for treating and preventing cancer; more particularly to treating or preventing malignant mesothelioma with antagonists of HMGB1

GRANT SUPPORT

Active Research Support:

DoD CA150220

10/01/16-09/30/18

(Yang PI, Carbone co-PI)

DoD Peer Reviewed Cancer Research Program Idea Award with Special Focus

Identification and validation of novel germline DNA variants associated to increased risk of malignant mesothelioma.

The objective of our research is to identify which are these genetic modifications that confer higher risk of developing mesothelioma. Identifying, among the millions of individuals exposed to asbestos (many belonging to the military), those at higher risk of mesothelioma, will allow us to implement personalized programs of screening and early detection and treatment of mesothelioma for these individuals.

DoD CA150671

07/01/16-06/30/19

(Yang Initiating-PI, Carbone, Mak, Pass, Kanodia Partner-PIs)

DoD Peer Reviewed Cancer Research Program Translational Team Science Award

HMGB1 and Its Isoforms As Biomarkers For Mineral Fiber Exposure and MM Detection

Our proposed studies will significantly enhance our knowledge of how mesothelioma develops, elucidate which isoform of HMGB1 is required at what stages of disease, and prospectively evaluate HMGB1 and its isoforms as a novel biomarker to distinguish between asbestos exposure and malignant mesothelioma.

NCI R01CA160715-01

07/01/11-06/30/17

The Role of HMGB1 in the Pathogenesis of Mesothelioma

(Yang, PI, 20% effort)

The overall goal of this project is to elucidate the mechanism(s) by which HMGB1 contributes to asbestos-induced initiation, maintenance and progression of mesothelioma and evaluate inhibition of HMGB1 as a novel strategy for the prevention and/or therapy of mesothelioma.

The V Foundation

10/31/12-10/30/16

HMGB1: A Biomarker for Mineral Fiber Exposure and Detection of Malignant Mesothelioma

(Yang, Basic Science PI, 15% effort; Carbone, Clinical PI; Pagano, Biostatistician PI)

The overall goal of this project is to study whether HMGB1 can be used as a serum biomarker for carcinogenic mineral fiber exposure and/or a biomarker to distinguish between lung cancer and mesothelioma.

United-4 A Cure Foundation

12/01/14-11/30/15

Novel Therapeutic Drug For The Treatment of Mesothelioma

(Yang, PI)

The overall goal of this project is to study the effect of novel therapeutic drugs for mesothelioma and to understand its biological functions and mechanisms.

NCI R01CA198138-01

07/01/15-06/30/20

Germline BAP1 Mutations and Malignant Mesothelioma: Mechanisms and Early Detection

(Carbone PI, Yang, co-I, 5% effort)

The overall goal of this project is to elucidate the mechanisms and genetic alterations that lead to mesothelioma in BAP1 mutant carriers, as well as to evaluate a biomarker that may be used to monitor the high-risk individuals for early detection of mesothelioma.

Pending Research Support:

NCI 1U01CA212213-01

12/01/16-11/31/21

Phase 3 Validation of Mesothelioma Blood Biomarkers

(Yang, co-PI, 10% effort)

This application will use the PLCO archive of mesotheliomas in order to determine whether the most studied mesothelioma candidate markers (HMGB1, MRP, Fibulin-3, and Slow Off Rate Aptamers (SOMAmers) have clinical utility in the pre-diagnosis/diagnosis/prognostication of this rare but socio-economically important neoplasm.

Completed Research Support:

DOD CDMRP PRCRP Career Development Award

09/30/13-01/29/16

Mesothelioma: identification of the key molecular events triggered by BAP1

(Yang, PI, 38% effort)

The overall goal of this project is to explore the possible interactions between the pathways activated by HMGB1 and those that are altered by BAP1 deletions. It is anticipated that these studies will lead to the identification of the cellular pathways that are critical in the process of asbestos carcinogenesis and mesothelioma growth, and thus provide specific targets to develop novel molecular therapeutic approaches.

Mesothelioma Applied Research Foundation

01/01/09-12/31/10 (Yang, PI)

Studies of TNF-a as a new Target for Human Malignant Mesothelioma Preventive and Therapeutic Strategies

The overall goal of this project is to further study the function of TNF-a and its related pathways in mesothelioma development and also in tumor progression, which will lead to the development of novel preventive and therapeutic strategies

United-4 A Cure Foundation

07/01/08-06/31/12

(Yang, PI)

Gene Expression Profiling Studies of Malignant Mesothelioma

The overall goal of this project is to perform the whole-genome expression profiling using the mesothelioma models that we have developed in order to identify the critical biological pathways and molecules that are involved in mesothelioma development.

Hawaii Community Foundation

09/24/08-09/24/11

(Yang, PI)

Mesothelioma Prevention and Early-detection Studies

The overall goal of this project is to identify novel preventive approaches and early detection strategies for asbestos-related thoracic malignancies, especially mesothelioma.